JOURNAL OF DAIRY SCIENCE

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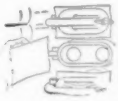

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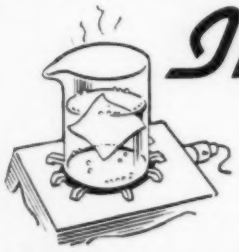

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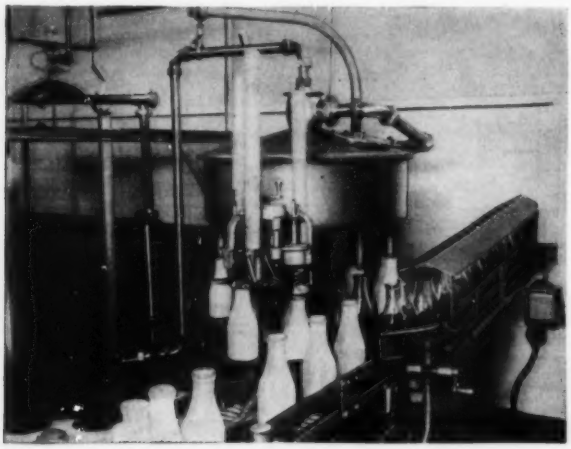

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIII DECEMBER, 1940 NUMBER 12

RELATION OF SURFACE TENSION OF RANCID MILK TO ITS INHIBITORY EFFECT ON THE GROWTH AND ACID FERMENTATION OF STREPTOCOCCUS LACTIS

N. P. TARASSUK AND F. R. SMITH

Dairy Industry Division, University of California

Koestler (1) first observed the inhibitory effect of rancid milk on the growth of *Streptococcus lactis* with the resultant delayed acid fermentation. He noticed that raw rancid milk will not become acid-coagulated even if kept at room temperature for several days. Using milk susceptible to rancidity, he made plate counts on the raw milk and on the milk pasteurized shortly after milking. Some samples were inoculated with *S. lactis* and some were not. The results indicated that the growth-arresting effect of rancid milk is very pronounced. According to Koestler, Roadhouse, and Lörtscher (2), rancid milk significantly inhibits (or slows) the growth of bacteria in milk in general and of *Streptococcus lactis* in particular. As Tarassuk (3) has shown recently, rancidity is one cause for the failure of lactic starters in acid coagulation of milk. The admixture of as little as five per cent of rancid milk in normal milk with the addition of the usual amount of starter gave an acid clot that was very weak. At the higher concentration of rancid milk, the acid coagulation was delayed for as long as eleven hours. Tarassuk found that delayed acid coagulation can be somewhat overcome by increasing the amount of starter added.

The frequency of rancidity in milk can be appreciated from recent contributions by Herrington and Krukovsky (4), Krukovsky and Herrington (5). In many dairy-manufacturing processes, on the other hand, a normal acid fermentation is of utmost importance. Explanation of the phenomenon involved in the inhibition of acid fermentation by rancid milk is, therefore, essential for a rational approach to this problem.

This report will furnish evidence for an explanation of why a rancid milk inhibits lactic-acid fermentation.

METHODS

The rancid milk used in these studies was one in which a lipase was naturally active—that is, a milk that would become rancid soon after milking without activation measures such as shaking or homogenization. The cow

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producing this milk was free from mastitis, Bang's disease, tuberculosis, and udder deformations.

The surface tension was measured at 20°–21° C. with the Cenco-du-Noüy tensiometer.

Hydrogen-ion concentrations were determined electrometrically with the Bailey hydrogen or quinhydrone electrode.

The bacteriological methods were essentially those described in *Standard Methods of Milk Analysis, sixth edition.*

EXPERIMENTAL RESULTS

I. *Surface Tension of Rancid Milk*

Doan and Minster (6) studying the changes in the physical and chemical properties brought about by homogenization of milk, showed that surface tension of homogenized milk is lowered by lipolysis. They attributed this surface tension lowering effect to the formation of appreciable amounts of surface tension active fatty acids. The hydrolysis of fat by lipase was accelerated by homogenization of raw milk. Tarassuk (3) working with milk in which lipase was naturally active has observed the same surface tension lowering effect. As the hydrolytic rancidity develops, the surface tension of milk decreases from a value of 49–51 dynes per cm. to a value of 39 dynes per cm., or even lower. This progressive lowering of surface tension has been confirmed in the present study. (See curve I, fig. 1.) If a milk

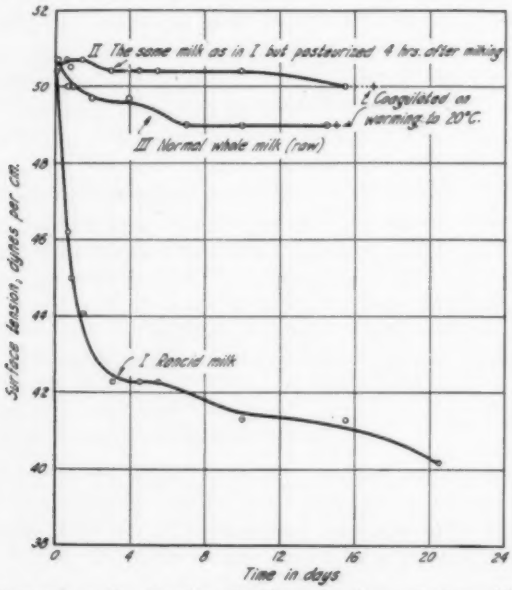

FIG. 1. Change in surface tension of milk on development of rancidity at 7° C.

known to become rancid is pasteurized within a few hours after milking, the surface tension upon aging remains practically constant (curve II, fig. 1).

As compared with a rancid raw milk, the surface tension of a normal milk changes very little throughout the aging period. It decreases as a rule, about one to two dynes per cm. during the first two days and remains fairly constant thereafter. This relatively slight decrease in surface tension may be attributed to (1) a change in the physical state of fat on cooling of milk, as shown by Bauer (7); (2) a slight lipolytic activity which according to Herrington and Krukovsky (4) manifests itself in practically all raw milk.

II. *Relation of Surface Tension of Rancid Milk to Its Acid Coagulation*

According to a well-recognized fact, surface tension of media is an important factor in the growth of organisms. Larson, Cantwell, and Hartzell (8) found that a depression of the surface tension would prevent surface growth of pellicle formers. Also, according to these authors, certain anaerobes could grow aerobically when the surface tension was lowered. Wolfe (9), in rather extensive studies on this subject, including several species of bacteria, found certain organisms to be more affected than others.

In the Lactobacillus group, the minimum surface tension at which the various species will grow is being used as a means of differentiation.

The work most pertinent to the subject under consideration is that done by Ayers, Rupp, and Johnson (10) on surface tension as affecting various strains of the streptococci. These authors studied several different depressants and several different surface-tension values. Seemingly, a surface tension below 40 dynes per cm. would be necessary to stop the fermentation reactions of *Streptococcus lactis* when inoculated into a carbohydrate medium. Unfortunately, surface tension has not been studied in relation to growth of *Streptococcus lactis* with a milk as a medium of growth.

(a) *Surface tension and acid fermentation of rancid milk aged at various temperatures*

In these experiments, milk containing a naturally active lipase was divided into three portions. The respective samples were aged at 7° C., 22° C., and 37° C. The development of rancidity and acid fermentation of the samples was observed by testing surface tension, titratable acidity, and hydrogen ion concentration at intervals of time.

In experiment 1, the samples were placed at their respective temperatures of holding three hours after milking. At this time the milk tested as follows: surface tension 49.7 dynes per cm., titratable acidity 0.12 per cent, and pH 6.83. In experiment 2, the milk was seven hours old, with surface tension of 45.5 dynes per cm., titratable acidity of 0.14 per cent, and pH of 6.69. Tested organoleptically, this milk was already definitely rancid.

Evidently, according to the data in table 1, the rate and extent of lipolysis was greatest when the milk was kept at low temperature. In all cases, the acid coagulation of milk was delayed. The coagulum formed under this

TABLE 1

Development of rancidity and acid fermentation of rancid milk aged at various temperatures

Experiment	Aging time	Aged at 7° C.			Aged at 22° C.			Aged at 37° C.		
		S. T.	T. A.	pH	S. T.	T. A.	pH	S. T.	T. A.	pH
number		*dynes/cm.*	*per cent*		*dynes/cm.*	*per cent*		*dynes/cm.*	*per cent*	
1	34 hrs.	41.6	0.13	6.73	43.4	0.15	6.69	46.2	0.55	5.38
	59 hrs.	41.3	0.15	6.68	44.8	0.23	6.10	46.5	0.60	5.24
	83 hrs.	liquid			weak, partial coagulation			weak, partial coagulation		
	6½ days	39.5	0.16	6.63						
	46 days	weak, partial coagulation								
2	28 hrs.	41.3	0.15	6.63	42.7	0.16	6.53	44.8	0.20	6.27
	66 hrs.							weak, partial coagulation		
	4 days	39.4	0.17	6.59	44.5	0.44	5.44			
	5 days				weak, partial coagulation					
	43 days	41.6	0.32	5.81						

condition is weak, often resembling a precipitate rather than a clot. In experiment 2, the milk aged at 22° C. had stood for over 4 days before acid clot appeared. The same milk aged at 7° C. failed to coagulate on standing as long as 43 days. At the end of this period, in fact, the pH of the milk was 5.81, the titratable acidity 0.32 per cent.

The data in table 1 definitely indicate that the acid fermentation of rancid milk is markedly inhibited; likewise, that the lower the surface tension, the slower the acid fermentation. At favorable growth temperatures, however, not only does the increased acidity check the further development of rancidity (as might be expected), but a rancidity previously produced is decreased, as shown by the rise in surface tension.

(b) *Surface tension and acid fermentation of rancid milk when inoculated with Streptococcus lactis*

In these experiments a portion of the milk containing active lipase was pasteurized at 60° C. for 30 minutes within 3–6 hours after milking. Samples of the pasteurized and raw milk inoculated with a pure culture of *Streptococcus lactis* were incubated at 30° C. The data in table 2 show definitely the inhibition of growth of *Streptococcus lactis* in part of a sample of milk in which lipase was allowed to remain active. These studies confirm also the previous suggestion that when the growth of *Streptococcus lactis* in rancid milk reaches a certain stage, the surface tension of milk increases.

(c) *The growth and acid fermentation of Streptococcus lactis in sterilized rancid milk and their relation to the surface tension of the milk*

That the phenomenon of delayed acid fermentation of rancid milk is

TABLE 2

Development of rancidity and acid fermentation in lipolytically active raw milk and in the same milk pasteurized. Both milks inoculated with Streptococcus lactis

Sample	Time of incubation	Plate count	Surface tension	Titratable acidity	pH	Remarks
	hours		*dynes/cm.*	*per cent*		
(a) Raw milk—4 hrs. after milking	immediately after inoculation	133,000	49.7	0.14	6.67	
(b) Milk (a) pasteurized	"	144,000	50.0	0.14	6.68	
(a) Raw milk	20	500,000,000	45.5	0.20	6.13	
(b) Pasteurized	20	1,180,000,000	50.0	0.25	5.90	
(a) Raw milk	28	.....	.....	.....	.....	Liquid Coagulated
(b) Pasteurized	28	.....	.....	.....	.....	
(a) Raw milk	33	.....	46.2	0.54	5.41	Liquid Coagulated
(a) Raw milk	35	.....	.....	.....	.....	

caused by a low surface tension of such milk is again demonstrated in the following experiments. Whole milk containing a naturally active lipase was allowed to stand in the cold until it became very rancid. During this time the surface tension decreased from 50.7 dynes per cm. to 41.3 dynes per cm. Using the rancid milk and fresh normal milk, the following samples were made: I. normal whole milk, II. normal whole milk + 0.1 per cent of diglycol laurate, and III. rancid whole milk. All samples were sterilized in an autoclave at 115° C. for 20 minutes. After cooling they were inoculated with *Streptococcus lactis* and incubated at 30° C. At frequent intervals during the incubation period, samples were examined to determine the numbers of bacteria, the titratable acidity, the surface tension, and the time of coagulation. The results appear in figures 2, 3, and 4.

The reason for inoculating the sterile samples of milk with *Streptococcus lactis* was as follows. If the surface tension is the factor in the phenomenon of delayed acid fermentation of rancid milk, then a sterile rancid milk inoculated with *Streptococcus lactis* should also exhibit the same phenomenon, provided that sterilization does not materially change the surface tension of rancid milk. Actually, we found that sterilization of rancid milk raises its surface tension only one to two dynes per cm. Furthermore, a normal milk whose surface tension is lowered by means other than lipolysis should behave like a rancid milk in respect to acid fermentation. It is for this reason that a diglycol laurate, a powerful surface-tension depressant oil,* was added to sample II.

As is evident from figure 2, the growth of *Streptococcus lactis* in milk

* It is advertised by a manufacturer as a non-toxic, edible oil.

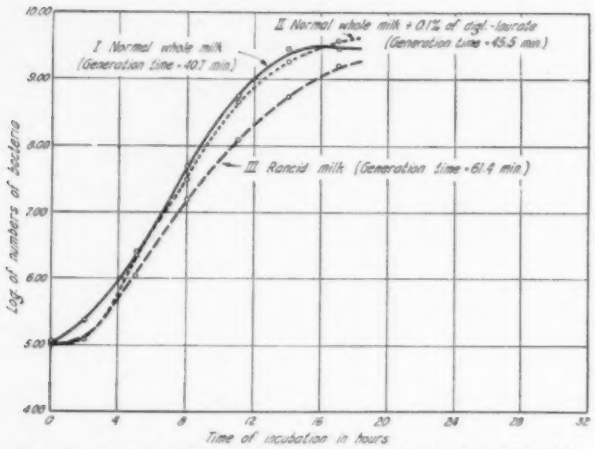
II Normal whole milk + 0.1% of digl.-laurate
(Generation time = 45.5 min.)
I Normal whole milk
(Generation time = 40.7 min.)
III Rancid milk (Generation time = 61.4 min.)
Log of numbers of bacteria
Time of incubation in hours

FIG. 2. Growth of *Streptococcus lactis* in sterilized rancid milk.

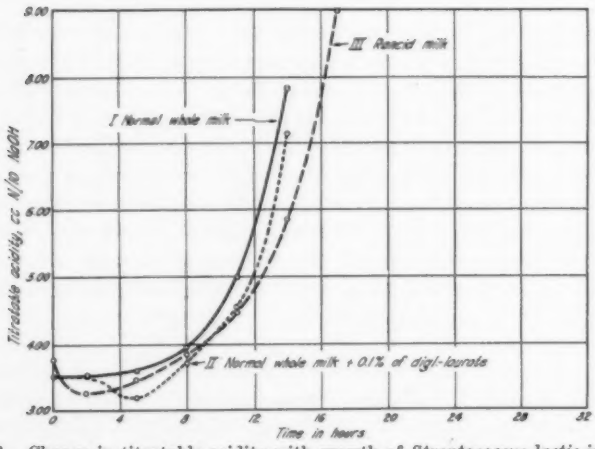

FIG. 3. Change in titratable acidity with growth of *Streptococcus lactis* in sterilized rancid milk.

with a low surface tension is markedly inhibited. This inhibition is true whether caused by the addition of a surface-tension depressant or by a low surface tension resulting from a hydrolysis of milk fat. As the growth curves of *Streptococcus lactis* show, when the surface tension is low there is a longer lag period, together with a marked increase in the generation time in the period of logarithmic growth. Along with the inhibition of growth one finds a marked delay in acid fermentation. This latter fact is shown by the curves depicting the change in titratable acidity presented in figure 3.

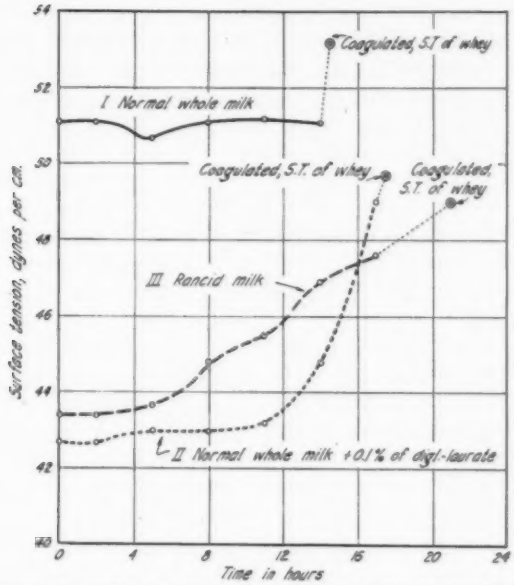

FIG. 4. Change in surface tension with growth of *Streptococcus lactis* in sterilized rancid milk.

The unexpected and most interesting detail in this graph is the decrease in titratable acidity in samples II and III during the first four to five hours of growth. The reason for this decrease becomes apparent after we study in figure 4 the change in surface tension brought about by growth of *Streptococcus lactis*. During the acid-fermentation period resulting from the growth of *Streptococcus lactis*, the surface tension of a normal milk remains essentially unchanged. On the other hand, in rancid milk and normal milk with depressed surface tension, the surface tension begins to increase after the first two hours of incubation. This increase becomes very appreciable during the logarithmic stage of growth; and when acid fermentation reaches the coagulation stage, the surface tension of these samples of milk approaches the surface-tension value of a normal milk. The change in surface tension, therefore, must come through a change in the depressant. In rancid milk, then, the fat acids responsible for the lowering of surface tension are utilized by *Streptococcus lactis* in the process of growth. Thus, a decrease in titratable acidity in the first stage of growth becomes self-apparent.

In a separate experiment in which lauric acid was used as a surface-tension depressant, similar results in respect to changes in surface tension and titratable acidity were obtained.

The ability of *Streptococcus lactis* to utilize certain fat acids in the process of growth and thus to raise the surface tension of rancid milk is

confirmed also by the following fact. The surface tension of whey obtained from rancid whole milk by a direct precipitation with acid is normally higher by two or three dynes per cm. than that of milk itself. In contrast, the surface tension of the whey obtained from a rancid milk as a result of *Streptococcus lactis* fermentation, may be as much as ten dynes per cm. higher than that of milk itself prior to fermentation.

CONCLUSIONS

1. Inhibitory effect of rancid milk on the growth and acid fermentation of *Streptococcus lactis* has been confirmed.

2. The inhibitory effect has been shown to result from a low surface tension of rancid milk.

3. Appreciable growth of *Streptococcus lactis* in rancid milk increases the surface tension of the milk. Under optimum conditions of growth in respect to temperature of incubation and initial number of organisms, this increase in the surface tension of rancid milk yields a final surface-tension value approaching that of normal milk. The change in surface tension apparently results from the utilization of surface-tension-lowering fat acids by *Streptococcus lactis* in the process of growth.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. G. A. Richardson for helpful suggestions and criticism of certain parts of the manuscript.

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AGE AS A FACTOR INFLUENCING BREEDING EFFICIENCY IN A DAIRY HERD*

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One of the objections to the exclusive use of proved sires in a dairy herd pertains to the difficulty of getting heifers safe in calf. The general opinion is that a greater number of services is required per conception in heifers when bred to mature bulls than when bred to younger bulls. Breeding efficiency in dairy cattle is of considerable economic importance. This is especially true where dairymen are making an effort to maintain a uniform production level, or attempting to have their cows and heifers calve during a certain period in order to establish a base production level that will meet the marketing situation prevalent in many milk sheds.

The data that have accumulated in the herd books of the West Virginia Agricultural Experiment Station have been studied with the hope that some light might be thrown on the influence of age on the breeding efficiency of dairy cattle. A preliminary report (1) indicated a smaller number of services per conception in heifers when bred to young bulls than when bred to older bulls. These results checked with the conclusions of Morgan and Davis (2) based on a similar study in the Nebraska Station herds. In order that more definite conclusions might be drawn in regard to this question, a further analysis was made of the data at hand with special reference to the influence of the age of the bull and the age of the female upon the number of services required per conception in dairy cattle.

PROCEDURE

The data for this study were taken from the herd books of the Dairy Department of the West Virginia Agricultural Experiment Station for the period from January 1, 1920 to August 1, 1938. Only those animals which had given birth to calves, or which were known to be breeders, were considered. All animals which were known to be non-breeders were discarded from the data and were not used in tabulating the results.

Since all of the Ayrshires and a large percentage of the cows of the other breeds were negative to the Bang's test no attempt was made to study the two groups separately and both were treated as one group.

The bulls ranged in age from one to fifteen years. The ages of the bulls were calculated to the nearest year. The ages of the females were considered on the basis of conception since the conception seemed to be more important than actual age in this study.

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The females were grouped by conceptions ranging from one to six. However, due to the small number of cows in the sixth group it was considered advisable to combine the fifth and sixth conceptions and treat them as one. The number of animals beyond the sixth group was so small it was not considered. The study included a total of forty-three males and seven hundred six females grouped as shown in table 1.

TABLE 1

Arrangement of total number of females according to breed and conception

Conception	Breed of cattle			
	Ayrshire	Holstein	Jersey and Guernsey	Total
First	420	131	80	631
Second	322	106	69	497
Third	205	71	48	324
Fourth	123	45	32	200
Fifth and Sixth	106	38	26	170

RESULTS

Table 2 is a summary of the results of using bulls of all ages on females of all ages. Where no consideration was given either to the age of the bull or to the age of the female to which he was mated, the heifers that were bred for first calving required a larger number of services per conception than did the females of any other group. After the first conception, however, the number of services per conception did not vary to a marked degree.

TABLE 2

Average number services per conception using bulls of various ages on females of all ages

	Conception					
	1st	2nd	3rd	4th	5th and 6th	Total
Number of Females	631	497	324	200	170	
Number of Services	1601	894	572	354	289	3710
Number of Conceptions	631	497	324	200	170	1822
Services per Conception	2.79	1.86	1.79	1.82	1.80	2.02

When the data are divided to show the influence of the age of both the bulls and the females a more detailed picture of the results can be seen. This material is presented in table 3. It is apparent that the age of the bull has a significant effect upon the number of services per conception in the case of heifers. Bulls under four years of age when bred to heifers showed a greater efficiency than bulls of any other age. In the case of the females bred for the second conception there was also a small but significant differ-

ence in favor of the bulls under four years of age. Beyond the second conception there was no significant effect of the age of the bull.

In the case of cows of all ages bred to bulls under four years of age there is not a marked difference in the number of services per conception, except in the case of uncalved heifers. In all but three of the eleven age intervals for bulls above four years, however, a larger number of services per conception was required in the case of first conceptions than for any other conceptions.

Figures 1 to 5 inclusive show the regression lines and the regression

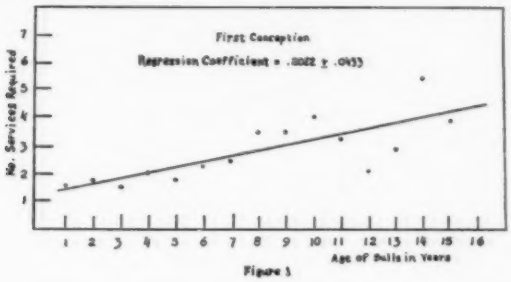

Figure 1

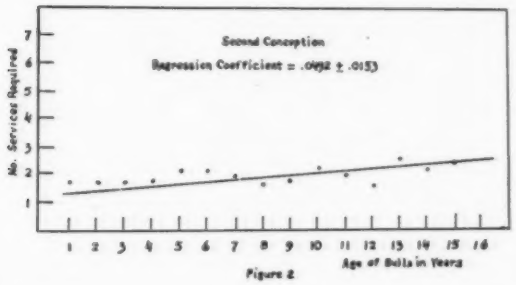

Figure 2

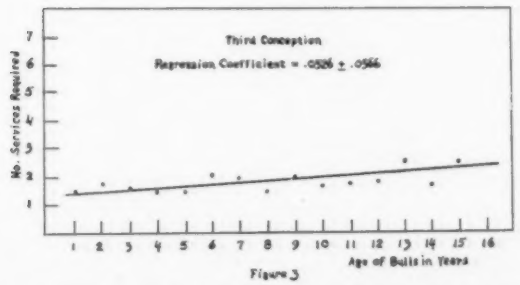

Figure 3

coefficients when the number of services for the various conceptions are plotted against the age of the bulls. The regression coefficients were plotted by the method of least squares.

The regression coefficients for figures 1 and 2 are highly significant. The regression coefficients for the remaining figures are not significant but the lines show a tendency for the breeding efficiency to decrease as the age of the bull increases. Figure 6 shows a comparison of the services required

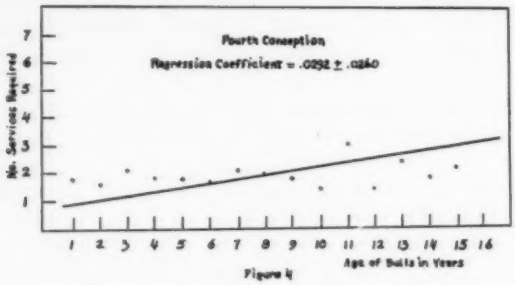

Figure 4

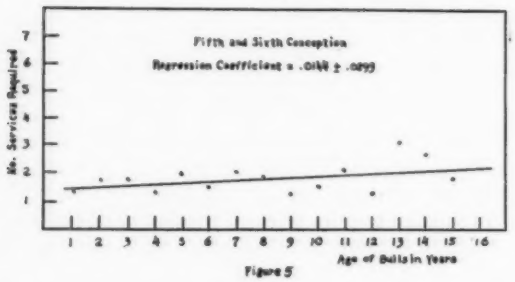

Figure 5

TABLE 3

Effect of age of bulls upon services per conception with females of different ages (conceptions)

Age of bulls	Conception 1st	2nd	3rd	4th	5th & 6th	Mean
1– 2 years ...	1.49	1.58	1.38	1.72	1.33	1.50
2– 3 "	1.62	1.45	1.76	1.57	1.76	1.63
3– 4 "	1.53	1.45	1.51	2.00	1.77	1.65
4– 5 "	1.97	1.58	1.46	1.58	1.22	1.56
5– 6 "	1.79	2.09	1.42	1.73	1.92	1.79
6– 7 "	2.23	2.04	2.00	1.53	1.57	1.87
7– 8 "	2.42	1.82	1.93	2.08	2.00	2.05
8– 9 "	3.56	1.61	1.41	1.84	1.81	2.04
9–10 "	3.50	1.72	1.96	1.72	1.20	2.02
10–11 "	4.06	2.06	1.60	1.25	1.53	2.10
11–12 "	3.27	1.88	1.77	3.00	2.00	2.38
12–13 "	2.00	1.52	1.83	1.25	1.20	1.56
13–14 "	2.97	2.52	2.52	2.25	3.14	2.68
14–15 "	5.50	2.17	1.68	1.76	2.66	2.76
15–16 "	3.90	2.30	2.58	2.00	1.80	2.57
Mean	2.79	1.85	1.79	1.82	1.79	

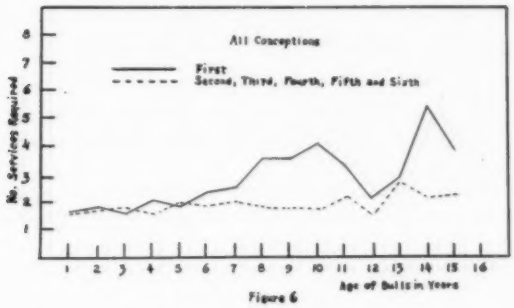
All Conceptions
First
Second, Third, Fourth, Fifth and Sixth
No. Services Required
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Age of Bulls in Years

Figure 6

for first conceptions with the average number of services required for conceptions at all ages.

TABLE 4

Analysis of variance of services per conception for animals in different age groups

Source of variation	Degrees of freedom	Sums of squares	Variance
Total	74	40.460	.546
Between conceptions	4	11.419	2.855**
Between ages of bulls	14	12.049	.861**
Discrepance	56	16.992	.303

Least significant mean difference between conceptions ..... .68
Least significant mean difference between ages ..... .40
** Highly significant.

SUMMARY

Seven hundred and six females and forty-three males are included in this study. A summary of their breeding records shows that when no consideration was given to the age of the bull the number of services required for the first conception was significantly higher than for the following conceptions. Uncalved heifers required a smaller number of services per conception when bred to bulls under four years of age than when bred to older bulls.

There was a gradual decrease in the breeding efficiency of bulls as they increased in age, but when used on females of all ages this decreased efficiency, when compared to the yearling age, did not become significant until the bulls reached an average age of six years. Bulls 13 years old and over were significantly less sure as breeders than bulls six years of age.

It appears from this study that the use of old bulls is an important factor in the breeding efficiency of dairy heifers, and that dairymen may be

justified in mating heifers with young bulls in order that prompt conceptions may result.

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A SEMIMICRO-KJELDAHL METHOD FOR THE DETERMINATION OF TOTAL NITROGEN IN MILK

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Recent research work in this laboratory pertaining to the nitrogen distribution in dairy products made it desirable to develop a practical method for determining small amounts of nitrogen.

A survey of the literature disclosed a number of methods designed for this purpose, but after careful consideration of the time factor, routine applicability and accuracy, the semimicro-Kjeldahl procedure used by Rowland (1) was chosen as the most practical method for the analysis of milk.

Rowland does not describe the distillation apparatus he used, but stated that direct steam distillation was utilized to liberate ammonia from the digested samples. He used selenium oxychloride in the digestion mixture because it markedly reduced the time for digestion, increased the amounts of nitrogen determined and improved the agreement of duplicates.

The results this author reported for the total nitrogen in milk are not compared with the Official Method (2), but he stated that duplicate determinations agreed within $\pm$ 0.2 per cent nitrogen.

The purpose of this experiment was threefold: (a) to design a semimicro-Kjeldahl apparatus, (b) to compare the efficiency of several digestion catalysts and (c) to compare both standard acid and boric acid as the ammonia receiving agents.

DESCRIPTION OF APPARATUS

The apparatus shown in figure 1 proved to be very efficient. The ammonia is liberated from the digested sample by direct steam distillation through a small distillation bulb and a 250 mm. condenser which has a pyrex center tube. Steam is furnished by heating a 3 liter flask containing distilled water slightly acidified with sulphuric acid.

REAGENTS USED

(1) *50 per cent Sodium Hydroxide Solution*

500 gms. U.S.P. NaOH, 500 ml. of distilled water and 125 gms. of sodium thiosulphate.

(2) *Standard Sulphuric Acid and Sodium Hydroxide Solution*

The acid and alkali solutions used for titrations were 0.02 N.

(3) *Boric Acid Solution*

A stock solution contained 1 pound of boric acid to 10 liters of distilled water. This solution was diluted (2 parts to 3 of water) and 25 ml. used to receive the ammonia.

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(4) *Indicator*

100 mg. of methyl red and 30 mg. of methylene blue dissolved in 60 ml. of 95 per cent ethyl alcohol and made up to 100 ml. with distilled water.

(5) *Catalysts*

Three catalysts were used in the following experiments:

(a) 0.2 gm. copper sulphate and 2 drops of selenium oxychloride.

(b) 0.14 gm. HgO and 2 drops of selenium oxychloride.

(c) 0.14 gm. HgO only.

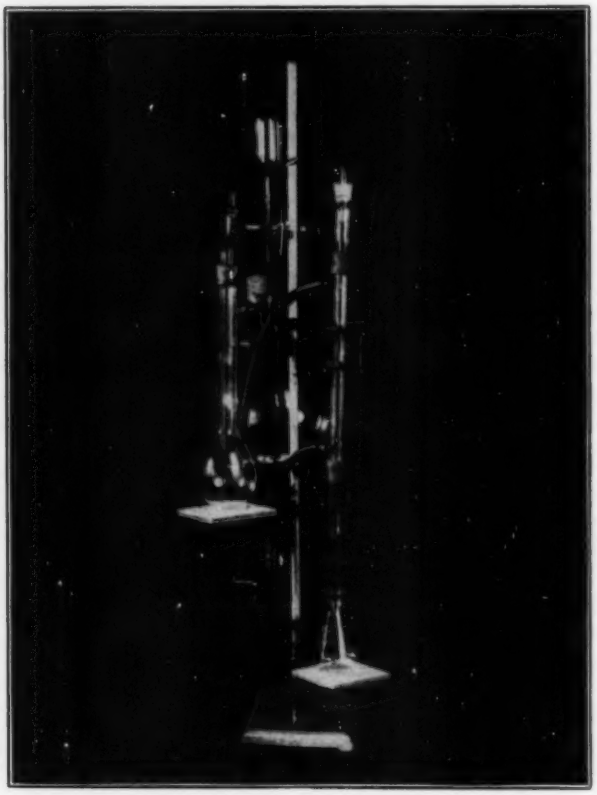

FIG. 1. Semimicro-Kjeldahl distillation apparatus.

PROCEDURE

Approximately five grams of milk, at room temperature, were weighed with a Mojonnier weighing cross and 5 gram pipets. The pipets were emptied into 100 ml. volumetric flasks and these filled to the mark with

distilled water. The samples were thoroughly mixed and 20 ml. of each pipetted into 300 ml. Kjeldahl digestion flasks. The different catalysts were added in the proportions mentioned above. Two gm. of sodium sulphate and 5 ml. of nitrogen-free sulphuric acid were then added and the samples containing selenium were digested over a moderate flame for 40 to 45 minutes or about fifteen minutes after becoming clear. The samples containing HgO only as a catalyst were digested for the same length of time but with a slightly hotter flame.

After digestion 50 ml. of distilled water was added to each sample. Fifteen ml. of the 50 per cent NaOH solution was added and the samples steam-distilled for approximately 10 minutes, the distillate passing into a 125-ml. Erlenmeyer flask containing 25 ml. of 0.02 N acid to which 3 or 4 drops of the methyl red—methylene blue indicator had been added. The indicator was added with a 0.1-ml. pipet. In some experiments 25-ml. of boric acid solution, containing the same amount of indicator, was used to receive the ammonia.

Just before starting a series of distillations a clean Kjeldahl flask containing a little distilled water was steam-distilled until the walls of the condenser and the distillation bulb were covered with moisture. This was done to prevent any loss of ammonia in the air discharged from the system at the beginning of the distillation.

All samples were distilled so that 60 to 70 ml. of the distillate collected in approximately 10 to 12 minutes. A 10-ml. buret graduated to 0.05 ml. was used for making these titrations. Duplicate blank determinations of nitrogen in the reagents were made with each set of samples.

The following experiments show the recovery of nitrogen from urea and the determination of protein ($N \times 6.38$) in various samples of milk. All results are compared with those obtained by the Official Kjeldahl-Gunning-Arnold Method (2) and this will be referred to as the Official Method.

The following tables show the recovery of nitrogen from urea using all three catalysts and with standard acid and boric acid as the ammonia receiving agents. For the semimicro-Kjeldahl 240 mg. of urea were dissolved in 500 ml. of distilled water and 25 ml. taken for a sample. For the Official Method 1.2 gm. of urea were dissolved in 500 ml. of distilled water and 25 ml. taken for a sample.

The Official Method gave a recovery of 27.50 mg. of nitrogen from the 25 ml. samples used in the determinations. On the basis of this recovery 5.50 mg. of nitrogen should be recovered from each semimicro sample. The percentage of nitrogen recovered in the following tables is computed on the basis of this value (5.50 mg. of nitrogen per sample).

The following samples were distilled into standard acid and back titrated with standard alkali.

TABLE 1

Recovery using 25 ml. of standard acid as the ammonia receiving agent and titrating with standard alkali

Se and $CuSO_4$	Recovery	Se and HgO	Recovery	HgO	Recovery
	per cent		*per cent*		*per cent*
5.46	99.27	5.44	98.90	5.38	97.82
5.40	98.18	5.34	97.09	5.47	99.45
5.43	98.73	5.11	92.90	5.45	99.09
5.36	97.45	5.52	100.36	5.36	97.45
5.50	100.00	5.34	97.09	5.40	98.18
5.45	99.09	5.39	98.00	5.57	101.27
5.46	99.27	5.42	98.55	5.40	98.18
5.48	99.64	5.50	100.00	5.45	99.09
5.40	98.18	5.43	98.73	5.47	99.45
5.49	99.82	5.47	99.45	5.32	96.73
Av. 5.45	99.09	5.40	98.18	5.43	98.73

TABLE 2

Recovery using 25 ml. of boric acid solution as the ammonia receiving agent and titrating direct with standard acid

Se and $CuSO_4$	Recovery	HgO	Recovery
	per cent		*per cent*
5.56	101.09	5.53	100.55
5.57	101.27	5.49	99.82
5.58	101.45	5.58	101.45
5.51	100.08	5.47	99.45
5.54	100.73	5.58	101.45
5.51	100.08	5.58	101.45
5.54	100.73	5.35	97.27
5.54	100.73	5.37	97.60
5.60	101.82	5.46	99.27
5.48	99.64	5.36	97.45
Av. 5.54	100.73	5.48	99.64

TABLE 3

Analysis of homogenized milk with boric acid as the receiving agent (Official Method—3.41 per cent protein)

Se and $CuSO_4$	*	Se and $CuSO_4$	*	HgO	*	HgO	*	HgO	*
3.38	−0.03	3.41	0.00	3.37	−0.04	3.44	+0.03	3.43	+0.02
3.34	−0.07	3.38	−0.03	3.31	−0.10	3.40	−0.01	3.44	+0.03
3.41	0.00	3.41	0.00	3.30	−0.11	3.44	+0.03	3.42	+0.01
3.40	−0.01	3.32	−0.09	3.42	+0.01	3.45	+0.04	3.44	+0.03
3.39	−0.02	3.40	−0.01	3.43	+0.02	3.42	+0.01	3.33	−0.08
3.27	−0.14	3.38	−0.03	3.35	−0.06	3.46	+0.05	3.43	+0.02
3.40	−0.01	3.40	−0.01	3.43	+0.02	3.42	+0.01	3.44	+0.03
3.30	−0.11	3.40	−0.01	3.43	+0.02	3.40	−0.01	3.45	+0.04
Av. 3.36	−0.05	3.39	−0.02	3.38	−0.03		3.43	+0.03	

* The difference between the semimicro values for total protein and the Official Method.

TABLE 4

Analysis of homogenized milk (Official Method—3.51 per cent protein)

Recovery using standard acid				Recovery using boric acid			
Se and $CuSO_4$	*	HgO	*	Se and $CuSO_4$	*	HgO	*
3.68	+0.17	3.38	−0.13	3.45	−0.06	3.53	+0.02
3.39	−0.12	3.42	−0.09	3.33	−0.18	3.49	−0.02
3.37	−0.14	3.46	−0.05	3.42	−0.09	3.50	−0.01
3.48	−0.03	3.45	−0.06	3.45	−0.06	3.52	+0.01
3.46	−0.05	3.42	−0.09	3.45	−0.06	3.50	−0.01
3.39	−0.12	3.39	−0.12	3.54	+0.03	3.44	−0.07
3.40	−0.11	3.44	−0.07	3.49	−0.02	3.51	0.00
3.45	−0.06	3.34	−0.17	3.51	0.00	3.52	+0.01
Av. 3.45	−0.09	3.41	−0.10	Av. 3.46	−0.06	3.50	−0.009

* See table 3.

Samples of abnormal milk were sent to the laboratory for complete analysis so it was possible to calculate the percentage of protein by difference in addition to making semimicro and macro analyses for protein. All three catalysts were used in these experiments and standard acid used to receive the ammonia. The samples in these experiments are all from the

TABLE 5

A comparison of different methods of determining protein

Experiment 1

Sample No.	Protein Semimicro Se & $CuSO_4$	Protein Official	Protein by difference		
	per cent	*per cent*	*per cent*		
587	3.28	2.98	3.34		
573	3.65	3.64	3.64		
746	3.18	3.23	3.20		

Experiment 2

Sample No.	Protein Semimicro Se & HgO	Protein Semimicro HgO	Protein Official	Protein by difference	
	per cent	*per cent*	*per cent*	*per cent*	
587	3.19	3.20	3.24	3.24	
573	3.65	3.65	3.61	3.61	
746	3.23	3.32	3.34	3.33	

Experiment 3

Sample No.	Protein Semimicro Se & $CuSO_4$	Protein Semimicro Se & HgO	Protein Semimicro HgO	Protein Official	Protein by difference
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
587	3.43	3.39	3.42	3.42	3.43
573	3.91	3.90	3.93	3.92	3.89
746	3.27	3.31	3.33	3.18	3.22

same cows but each experiment is a different set of samples. The results tabulated in table 5 are the averages of duplicate determinations.

The data presented in table 6 show the approximate digestion time required for each catalyst. The gas burners were adjusted to a moderate flame and not changed until the digestion of all the samples was completed. Samples were digested 15 minutes after clear and standard acid used as the receiving agent.

TABLE 6

The effect of the catalyst on the digestion time required (Official Method—3.43 per cent protein (Homogenized milk))

Catalyst	Per cent protein found after digesting				
	20 min.	30 min.	40 min.	50 min.	60 min.
Se & $CuSO_4$	.....	3.39	3.44	3.44	3.44
Se & HgO	3.41	3.45	3.46	3.45	3.41
HgO	.....	3.39	3.44	3.46	3.43

Some investigators (3, 4) report the loss of ammonia when samples containing selenium are subjected to a prolonged digestion. In the following experiment (table 7) four burners were adjusted so that each burner gave a hotter flame than the preceding one. The first sample, shortest digestion time, was heated to extremes. All samples were digested until 15 minutes after clear. Boric acid was used as the receiving agent.

TABLE 7

The effect of the catalyst on the digestion time required when the intensity of the heat was varied (Official Method—3.41 per cent protein (Homogenized milk))

Se & $CuSO_4$		Se & HgO		HgO	
Digestion time	Protein	Digestion time	Protein	Digestion time	Protein
min.	*per cent*	*min.*	*per cent*	*min.*	*per cent*
20	3.37	25	3.34	30	3.40
30	3.39	30	3.40	32	3.43
40	3.36	38	3.43	40	3.37
55	3.41	38	3.43	47	3.44

In the last two results in the columns above the burners were adjusted about the same as the adjustment used in the regular digestion procedure. There seems to be little effect by heating the samples excessively.

The catalyst containing selenium oxychloride and HgO seems to be slightly more efficient than the other catalysts in this and especially in the previous experiment.

DISCUSSION

In the data presented no distinct differences can be noted in the nitrogen and protein values obtained by using the various catalysts.

Rowland (1) in his original report stated that the addition of selenium to the digestion mixture increased the amount of nitrogen determined, but his comparison was made between copper sulphate with and without the addition of selenium.

It is not surprising that the addition of selenium increased the nitrogen values because copper sulphate seems to be inefficient for digesting milk protein. The authors have found that copper sulphate alone is a slower catalyst than HgO or metallic mercury and it has a tendency to cause low results for total nitrogen if the same digestion time is used for all three catalysts.

Lauro (5) used selenium alone as a catalyst because of its digestion efficiency. He also considered it a desirable catalyst because no precipitating agent is required. Most of the data pertaining to the use of selenium as a catalyst has been obtained by the analysis of cereal grains or their products and these results are somewhat conflicting (4, 6, 7).

Poe and Nadler (8) state that a combination of Cu, selenium and Hg effects the greatest saving of time for digestion. Prince (9) claims selenium alone has no advantage over mercury but when they are used together the digestion time is lessened one half. Osborn and Krasnitz (10) have somewhat the same opinion. They found that mercuric oxide and selenium is a more effective catalyst than selenium and copper sulphate. They conclude that when the digestion period is extended the danger of the loss of nitrogen increases in the following order: mercury, selenium and copper sulphate, and mercuric oxide and selenium. They believe that the danger from the loss of nitrogen may be reduced by using larger amounts of acid.

Davis and Wise (6) logically state that the indications favor a lower result with the use of selenium but this may be compensated for by careful research by each collaborator into the time of digestion, intensity of heat applied and the amount of sulphate used in the digestion.

The above discussion would lead to the conclusion that selenium would probably not be as universally used as metallic mercury or mercuric oxide. The literature for the most part, in spite of conflicting evidence, indicates that low results are experienced with the use of selenium or its combinations.

Because of this criticism of selenium the writers prefer to use HgO. This gives results as satisfactory as any of the selenium combinations.

Rowland used 0.02 N acid to receive the ammonia and boiled the distillates to remove carbon dioxide. After cooling, the samples were titrated with 0.02 N NaOH using 0.1 per cent methyl red solution as the indicator.

This procedure is not well adapted to routine analysis and the endpoint in the titration is not entirely satisfactory because it is not well defined.

The use of the boric acid solution as the receiving agent for the ammonia is preferred because it eliminates boiling the samples to remove carbon dioxide. The solution does not have to be measured accurately; it elimi-

nates the use of the dilute NaOH which requires frequent restandardization and is very sensitive to carbon dioxide. The direct titration with standard acid also simplifies calculations. In the previous experiments slightly higher and more consistent results have been obtained with the boric acid solution than with the standard acid and alkali.

Methyl red is not satisfactory for the direct titration of boric acid because the endpoint is indefinite. The methyl red-methylene blue indicator gives a distinct endpoint and has the advantage that the two components of this indicator may be adjusted so that the end point will satisfy the individual analyst. This combination indicator is also satisfactory if standard acid is used as the ammonia receiving agent.

As mentioned under "Procedure" (page 2) a flask containing distilled water was steam distilled before starting the samples to prevent the loss of ammonia. Redemann (11) claims that some of the error in the semimicro determinations is due to the loss of ammonia in the air forced out at the beginning of the distillation of a sample. For this reason the above procedure was adopted. It seemed reasonable to expect no loss of ammonia if the system contained moisture because of the water solubility of this compound and the small amount of nitrogen present in the semimicro sample (0.5 per cent approximately). This theory was checked by using some of the urea solution used in table 1 and 25 ml. of distilled water as the ammonia receiving agent. In the following samples selenium and copper sulphate were used as the catalysts. *N Recovered,* Mg 5.57, 5.55, 5.46, 5.52, 5.55, 5.48, 5.37, 5.47, 5.62, 5.55—Av. 5.51.

These results prove that very little if any of the ammonia is lost if some moisture is present on the walls of the system at the beginning of the distillation.

The semimicro method presented may be modified in many ways by the analyst and still give accurate results; however, its main advantage is the saving of time and the reduced cost of reagents per determination.

SUMMARY

1. A semimicro-Kjeldahl method and apparatus is described.

2. Mercuric oxide is recommended as a catalyst with boric acid as the ammonia receiving agent and methyl red-methylene blue as the indicator.

3. The semimicro method was found to check very closely with the Official Method.

4. The semimicro method is useful for determining small amounts of nitrogen, it is time saving, well adapted to routine analysis, and reduces the cost of reagents per determination.

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A STUDY OF FRESH AND FROZEN PLAIN, SUPERHEATED, AND SWEETENED CONDENSED SKIMMILK FOR ICE CREAM[1]

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INTRODUCTION

A method of storing milk solids in the form of frozen condensed skimmilk has been tried in the University of Nebraska Creamery and in general has proved successful. However, there have been times when the protein in the condensed skimmilk, which has been held frozen in storage, has become precipitated and gelation has occurred. These changes in the protein have not proved serious, but if the frozen condensed skimmilk is to be sold, it is necessary to reprocess it, which involves added expense and increases the cost of the milk solids.

In the freezing of milk and cream, viscosity as well as the factor of time plays a significant part in limiting the aggregation of colloidal particles. In the case of frozen cream to which sugar has been added, the point where viscosity limits the destabilizing effect of freezing is reached early and aggregation of the colloidal particles is reduced. On the basis of such an explanation it is reasonable to assume that similar results could be obtained by adding sugar to condensed skimmilk which is to be held frozen in storage. It has been shown by Pyenson and Dahle (12) that superheating of condensed skimmilk to 180° F. for 20 minutes brings about an increased stability of the proteins, as measured by the alcohol number, and that the large increase in viscosity due to superheating is associated with the coagulation of calcium caseinate and is not due to hydration. With this in mind, it was desired to include superheated condensed skimmilk in this study to observe the effects of freezing and storing on this type of condensed skimmilk. It seemed logical to believe that if condensed skimmilk was to be stored during the season of surplus and used during shortage, a three-months storage period would be sufficient, and it was upon this basis that the problem was studied.

The purpose of this problem was to study the effects of freezing and storing upon plain condensed skimmilk, superheated condensed skimmilk and sweetened condensed skimmilk, and to compare the advantages of one type over another as a possible means of storing serum solids for ice cream manu-

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facture. Any defects in the frozen condensed skimmilk which might add to the difficulties of incorporation in the ice cream mix, or which might require the condensed to be reprocessed, were particularly observed, as were any changes in the quality of the finished ice cream. Changes in the whipping ability of the ice cream mixes and any other variations resulting from the use of the three types of fresh and stored frozen condensed skimmilk were carefully observed.

REVIEW OF LITERATURE

Webb and Hall (17) found that fresh whole milk could be pasteurized, condensed to one-third its weight, canned and frozen without any detrimental effects to the body or flavor of the product. Such a milk, if held at a temperature below – 13° (8.6° F.), could be thawed and reconstituted with cold water to yield a product similar to normal fresh milk at any time up to the fourth week of storage. They stated that high heat treatment, long-time heat treatment or high storage temperature shortens the storage period in which the milk is free from casein precipitation.

Doan and Featherman (7) found that in concentrating and freezing milk for storage purposes, a degree of concentration of approximately three to one appears to give the most satisfactory results. Milk of such a concentration could be stored up to 12 weeks and still produce a normal reconstituted product.

Anderson and Pierce (1) showed that holding milk at – 25.7° C. (– 14° F.) gave no precipitated protein until the third month of storage and was usable until the sixth month. Milk held at – 12.5° C. (10° F.) showed some protein precipitation at the end of the second month, and at the end of the fifth month practically all of the protein had precipitated. Referring to the work of Webb and Hall, Doan and Baldwin (6) stated that freezing, itself, has no measurable effect upon protein dispersion and that holding milk in the frozen condition for several weeks or months is required to cause aggregation, denaturation, or instability of the proteins. Mack (8) found that the proteins of ice cream mixes made from frozen cream were less stable than those of the mixes made from fresh sweet cream, as measured by the protein stability test used by Doan (3).

While studying factors affecting the bound-water content of some dairy products, Dahle and Pyenson (2) found that holding skimmilk for long periods of time in the frozen state had no great effect upon alcohol stability of the proteins, but with condensed skimmilk destabilization of the proteins occurred. The condensed skimmilk increased in bound water when stored in a frozen condition for 25 days but after 57 days a marked precipitation had occurred. Doan (5) concluded that when frozen condensed whole milk is stored more than 8 weeks, the proteins become denatured and gelation occurs when the frozen condensed milk is thawed. Munkwitz, Berry, and

Boyer (9) stated that "freezing of milk causes a partial precipitation of milk solids, albumen being precipitated in the greatest amount, followed in order by lactose, total protein, ash, casein, total solids, and fat. With the exception of fat, the amount of precipitation of solids increases as the length of time of freezing increases."

Openlander and Erb (10) found that condensed skimmilk, when properly frozen and stored, could be used as a satisfactory source of serum solids in ice cream. The first noticeable defect that occurred in the ice cream made from frozen condensed skimmilk was a curdled appearance on melting. This defect could be easily corrected by the use of 0.3 per cent sodium citrate added to the mix at the pasteurizer. They also found that frozen condensed skimmilk testing 27 to 30 per cent total solids produced an ice cream that was less curdy than ice cream made from frozen condensed skimmilk testing 40 per cent total solids.

Reichart and Corley (15) have recently reported that frozen condensed skimmilk stored at −17.8° C. (0° F.) can be used satisfactorily as a source of serum solids in ice cream manufacture but that a storage period exceeding six months is not advisable. Frozen condensed skimmilk, after storage for four or more months, exhibited evidence of gelation upon melting, indicating that a denaturation of the protein had taken place during storage. However, the appearance of the final ice cream mix was not affected and no trouble was experienced in processing the mix. No consistent differences were noted in the whipping ability of the ice cream mixes nor in the flavor, body, and texture of the finished ice cream, but the ice cream made from the frozen condensed skimmilk melted down approximately twice as fast as that made from fresh condensed skimmilk.

According to Ramsey (13) the proteins in the ice cream mix should be in such a condition that they will absorb moisture readily. He stated that fresh plain condensed skimmilk or superheated condensed skimmilk is the best source of serum solids for ice cream and that sweetened condensed skimmilk and skimmilk powder can be used with fairly good results. Frozen condensed skimmilk is unsatisfactory because the proteins are denatured by freezing.

Tracy (15) and Tracy and Hahn (16) reported that the use of superheated condensed skimmilk in ice cream mixes will likely increase the viscosity of the mix somewhat over that of an ice cream mix containing plain condensed milk. They stated that there is no benefit from the use of superheated condensed skimmilk in ice cream manufacture so far as the time required to reach 100 per cent overrun is concerned. However, when a higher maximum overrun is desired, it may be used to an advantage.

Whitaker and Hilker (18) found that ice cream made from plain condensed skimmilk was less smooth, slightly weaker in body, and had a slightly less cooked flavor than ice cream made from superheated condensed skim-

milk. Using the sales-preference method of comparison, Williams and Hall (19) found that superheated condensed skimmilk produced a better quality of ice cream than did plain condensed skimmilk.

Pyenson and Dahle (12) stated that superheating of condensed skimmilk resulted in increased stability of the proteins, as measured by the alcohol test, and that the change in viscosity obtained by superheating was not due to hydration but to coagulation of the proteins.

METHOD OF TREATMENT

Preparation and Study of the Condensed Skimmilk. The three types (plain, superheated, and sweetened) of condensed skimmilk used in this study were prepared at three different times of the year (October 12, 1938; December 29, 1938; and February 11, 1939) to increase the scope of the data rather than to observe seasonal variations. The three types of condensed skimmilk prepared at any given period were made from the same lot of raw whole milk and processed in as nearly the same manner as possible.

For the manufacture of the three types of condensed skimmilk, approximately 350 gallons of skimmilk were taken immediately after separation, from pasteurized whole milk. Two-thirds of this skimmilk was heated to a temperature of 150° F. for forewarming and the remaining one-third was held cold, to be used later (approximately four hours) in preparing sweetened condensed skimmilk.

The skimmilk which had been forewarmed to 150° F. was then drawn into a Rogers 26-inch stainless steel vacuum pan and condensed to approximately 30 per cent total solids, under a vacuum of about 25 inches. One-half of the plain condensed skimmilk was drawn from the pan, cooled to about 55° F. a sample taken for analytical purposes and the remainder held at 45° F. until prepared for storage or used in an ice cream mix.

After obtaining about 25 inches of vacuum, the remaining portion of condensed skimmilk in the pan was superheated according to the method used by Doan (4). The temperature of the milk was raised to 180° F. and held for a sufficient length of time (five to fifteen minutes) to cause the desired increase in body as determined by striking and noting thickness. During this time the vacuum dropped from 25 inches to 10 to 13 inches. When the desired thickness was obtained the vacuum pump was started and the superheated condensed skimmilk was cooled in the pan to about 120° F., drawn from the pan and cooled to about 55° F. in a 50-gallon coil vat using ice water as the cooling medium. A sample was taken and the remainder was placed at 45° F. and held until prepared for storage or used in an ice cream mix.

The remaining one-third of the original skimmilk, which had been held cold, was preheated to 150° F. for 20 minutes. An amount of sugar to give one pound of sugar per pound of milk solids was added and the sweetened

skimmilk condensed to approximately 60 per cent total solids (30 per cent milk solids and 30 per cent sugar) under a vacuum of about 25 inches, drawn from the pan and cooled to about 55° F. with a small brine-cooled surface cooler. A sample was taken and the remainder was then placed at 45° F. and held until prepared for storage or used in an ice cream mix.

After the fat and total solids content of the condensed skimmilk had been determined by the Mojonnier procedure, the plain and superheated condensed skimmilk was standardized with distilled water to 30 per cent total solids. The sweetened condensed skimmilk was not standardized because in all cases the total solids content was slightly less than the desired 60 per cent.

The approximate amount of each type of condensed skimmilk needed for 300 pounds of ice cream mix was weighed into new five-gallon lard tins and stored at approximately 0° F. Three cans of each type of condensed skimmilk were put into storage at each period, one to be taken out at the end of each month for three months and made into an experimental ice cream mix.

As each monthly batch of condensed skimmilk was withdrawn from storage and thawed (at room temperature for 24 hours), observations were made for possible changes in physical structure or presence of "off-flavors" that might have developed during storage. After complete thawing, samples were taken from each type of condensed skimmilk for analytical purposes.

Preparation of the Ice Cream Mixes. On the day following the preparation of the condensed skimmilk and at the end of each month of storage three batches of ice cream mix, 300 pounds each, were made, using each of the three types of condensed skimmilk as the source of added serum solids. During this study a total of 35 ice cream mixes was prepared.

Other ingredients used in preparing the ice cream mixes were fresh sweet cream containing 34 per cent butterfat, skimmilk, sugar, corn sugar, and gelatin in quantities to give the following composition: butterfat, 14 per cent; serum solids, 10 per cent; sucrose, 13.5 per cent; corn sugar, 1.5 per cent; gelatin, 0.25 per cent. Each ice cream mix was pasteurized at 160° F. for a period of 20 minutes, homogenized in a two-stage 100-gallon-per-hour Manton Gaulin homogenizer at a total pressure of 3,000 pounds per square inch, with 500 pounds pressure on the second stage, and cooled over a surface cooler to approximately 40° F. and held below 45° F. for 20 to 24 hours.

Freezing the Ice Cream Mixes. The ice cream mixes were frozen in a Creamery Package 40-quart horizontal direct-expansion freezer. Before the experimental ice cream mixes were frozen, a preliminary batch was frozen in order to have the freezing conditions as nearly the same as possible. The mixes were frozen in duplicate and in succession, the ice cream mix containing the plain condensed skimmilk always being first, followed in order by the ice cream mixes containing superheated and sweetened con-

densed skimmilk. The temperature of the ice cream mix at the beginning of the freezing process, the temperature of the refrigerant, and the time required to freeze the ice cream mixes to 24° F. were recorded. Overrun readings were taken immediately after the refrigerant was shut off and at minute intervals thereafter until the overrun had reached a maximum.

Method of Taking Ice Cream Samples. Samples were obtained by filling quart Sealright containers directly from the freezer as soon as an overrun reading of 100 per cent was obtained, and immediately transferred to the hardening room held at approximately 0° F.

Analytical Procedure. The butterfat and total-solids determinations were obtained in duplicate by the Mojonnier method.

Solubility of the condensed skimmilk was determined by standardizing 200 grams to 9 per cent milk solids with distilled water at 70° F., mixing for one minute on an electric Hamilton-Beach Malted Milk Mixer and centrifuging a 25 ml. portion for 15 minutes in a Hart Casein test bottle at normal Babcock speed. The supernatant liquid was removed by means of a rubber siphon tube to within 2 or 3 ml. of the sediment and the test bottle refilled to the 25 ml. mark with distilled water. The test bottles were then shaken to dislodge the sediment in the bottom of the bottle and again centrifuged for 15 minutes. Each sample was represented by duplicate tests and the degree of solubility rated relatively as the number of smallest graduations on the Hart Casein test bottle, according to the amount of sediment collected in the bottom of the test bottle.

Protein stability of the ice cream mixes was determined by the alcohol number method used by Pyenson and Dahle (11). The titratable-acidity of the ice cream mixes was obtained by weighing nine grams of the sample at room temperature into a 125 ml. Erlenmeyer flask, adding 9 ml. of boiled distilled water, five drops of a one per cent alcoholic solution of phenolphthalein and titrating to the first definite and relatively permanent shade of pink with N/10 sodium hydroxide.

The pH of the ice cream mixes was determined at 25° C. (77° F.) by means of a quinhydrone electrode and a Leeds and Northrup Type K potentiometer.

Viscosity of the ice cream mixes was measured in triplicate at 5° C. (41° F.) with a Gramercy model MacMichael viscometer, using a 100 ml. sample and a No. 30 wire. The average of the three determinations was recorded as the viscosity in MacMichael degrees.

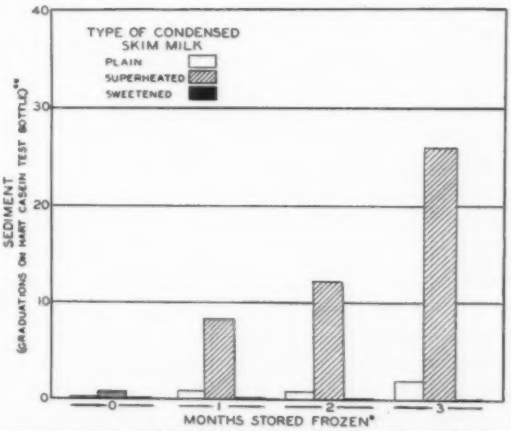
Judging the Ice Cream and Determining Melting Quality. The quart samples of ice cream were removed from the hardening room about 10 days after freezing and were cut in half. One pint of each sample was scored for flavor, body, and texture, and the remaining pints were set upon one-fourth-inch-mesh wire, resting on the rim of a heavy five-inch glass funnel with the end leading into a previously weighed 100 ml. graduated cylinder.

The number of minutes required for 100 ml. of melted ice cream to collect in the cylinder in a room held at 60° to 62° F. were recorded. The character of the melted ice cream as to smoothness and amount of foam was observed.

EXPERIMENTAL RESULTS

Physical Appearance of the Three Types of Condensed Skimmilk as Affected by Freezing and Storing. Comparisons were made concerning the body and physical appearance of each type of fresh condensed skimmilk and again at the end of each storage period (table 1). The superheated condensed skimmilk was changed most in physical appearance as shown by the slight curdy appearance and whey separation at the end of one month in storage, increased protein precipitation after two months, and marked precipitation after three months. The plain condensed skimmilk, which had been prepared in October, evidenced slight whey separation after two months in storage but was not criticized otherwise during this study. Since this defect did not occur in the plain condensed milk prepared in December and February, the defect may be ascribed to the quality of the original milk from which the October batch was prepared. Except for a slight sandy condition, there was no change in the physical appearance of the sweetened condensed skimmilk after three months in storage.

Fresh plain, and sweetened condensed skimmilk were almost completely



GRAPH 1. Comparative solubility of condensed skim milks. Fresh and stored frozen for the periods of time indicated.

* Average data from 3 series with the exception of 3 month period which is an average of only 2 series.

** Solubility is expressed as the number of smallest graduations on the graduated scale of the Hart casein test bottle.

soluble, and but very slightly more soluble than the fresh superheated condensed skimmilk. After one month in storage, the solubility of the plain condensed skimmilk decreased and continued to decrease very slowly up to the end of three months in storage. The solubility of the superheated condensed skimmilk decreased rapidly during one month in storage and continued to decrease rapidly during the storage period. The sweetened condensed skimmilk was almost completely soluble after three months in storage.

TABLE 1

Observations on the appearance of the condensed skimmilk, fresh and after thawing

Type of condensed skimmilk	Age (months frozen)	Date condensed		
		Oct. 12, 1938	Dec. 29, 1938	Feb. 11, 1939
Plain	0 (Fresh)	good	good	good
	1	sandy	good	good
	2	sandy sl. whey	good	good
	3		good	good
Superheated	0 (Fresh)	good	good	good
	1	sl. whey sl. curdy	sl. whey sl. curdy	sl. whey sl. curdy
	2	whey, curdy sl. gel.	sl. whey sl. curdy	sl. whey curdy
	3		curdy, gel., whey	sl. whey curdy
Sweetened	0 (Fresh)	good	good	good
	1	sl. sandy	sl. sandy	good
	2	sl. sandy	sl. sandy	good
	3		sl. sandy	good

Chemical and Physical Characteristics of the Ice Cream Mixes as Affected by the Three Types of Fresh and Stored Frozen Condensed Skimmilk Protein Stability. There was no consistent change in the protein stability (alcohol number) of ice cream mixes made with condensed skimmilk which had been stored frozen one month when compared to those prepared with the fresh product (table 2). However, mixes made with stored frozen superheated condensed skimmilk at this period showed less change than mixes made with plain or sweetened condensed skimmilk. It was also noticed that the protein stability of ice cream mixes, made with each type of condensed skimmilk which had been stored frozen two months, was consistently greater than that of mixes made with fresh condensed skimmilk or condensed skimmilk which had been stored frozen for one and three months. There was no apparent relationship between the solubility of the condensed skimmilk and the alcohol number of the ice cream mix in which it was used.

Titrable Acidity. There was very little difference in the titrable acidity of the ice cream mixes made with the three types of fresh condensed skim-

TABLE 2

Comparison of some chemical and physical properties of ice cream mixes, made from fresh and stored frozen condensed skimmilk

Condensed skimmilk				Ice cream mix											
Type and age	Solubility			Total solids			Butterfat			Viscosity†			Alcohol No.‡		
	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*
				%	%	%	%	%	%						
Plain															
Fresh	0	0	0	38.96	39.14	39.13	14.01	14.02	13.99	53	110	67	5.5	5.5	5.5
1 mo.	1	1	0	39.61	39.21	39.27	14.24	14.15	14.01	69	92	79	5.0	5.5	6.0
2 mos.	1	1	0	39.24	39.34	39.31	13.95	14.12	14.12	98	93	73	6.0	7.0	6.5
3 mos.	....	2	2	.....	39.19	39.38	.....	13.99	14.07	.....	67	92	....	6.0	5.5
Superheated															
Fresh	0	1	2	38.96	39.19	39.35	14.00	14.01	14.01	49	98	114	5.5	5.5	5.5
1 mo.	6	11	8	39.55	39.24	39.11	14.23	14.20	14.00	104	103	125	5.5	5.5	5.5
2 mos.	10	10	16	39.32	39.14	39.43	14.02	14.04	14.09	104	82	101	5.5	7.0	6.5
3 mos.	....	20	32	.....	39.38	39.40	.....	14.14	14.11	.....	203	84	....	6.0	5.5
Sweetened															
Fresh	0	0	0	39.31	39.46	39.21	14.02	14.16	14.02	53	101	86	6.0	6.0	3.5
1 mo.	0	0	0	39.31	39.17	39.01	14.05	14.21	13.98	112	75	91	5.0	5.5	5.0
2 mos.	0	0	0	39.44	39.42	39.40	14.28	14.15	14.12	118	67	63	6.0	6.0	6.5
3 mos.	....	0	0	.....	38.95	39.37	.....	14.05	14.02	.....	78	65	....	5.5	5.5

* Indicates date of making the condensed milk: I—Oct. 12, 1938; II—Dec. 29, 1938; III—Feb. 1, 1939.
† Viscosity is expressed in MacMichael degrees.
‡ Alcohol number is the ml. of 95% alcohol required to give the first sign of precipitation in a 5 ml. sample.

milk (table 3). The titratable acidity of the ice cream mixes made with the three types of stored frozen condensed skimmilk was in nearly every case equal to or less than the titratable acidity of mixes made with the fresh condensed skimmilk, thus indicating that freezing and storing condensed skimmilk for periods up to three months did not increase the titratable acidity of the ice cream mix in which it was used.

Effect on pH. The pH of ice cream mixes made with fresh sweetened condensed skimmilk was in every case slightly lower than the pH of ice cream mixes made with fresh plain and fresh superheated condensed skimmilk. This variation might be explained in part by the fact that even though the three types of condensed skimmilk were prepared from the same original batch of raw milk, the skimmilk used in preparing the sweetened condensed skimmilk was always subjected to a holding period of approximately four hours at 45° F. before it was drawn into the condensing pan. The pH of the ice cream mixes was always within the range of 6.2 and 6.4 and the differences were not significant as far as the type of fresh and stored frozen condensed skimmilk is concerned

Effect on Viscosity. Viscosity determinations show (table 2) that ice cream mixes made with superheated condensed skimmilk were but slightly more viscous than ice cream mixes made with plain and sweetened condensed skimmilk, indicating that the high viscosity of the superheated condensed skimmilk did not carry over appreciably into the ice cream mix. The viscosity of the ice cream mixes was not consistently affected by the freezing and storing of the three types of condensed skimmilk used.

Whipping Ability. Conclusions drawn from average whipping curves indicate that the ice cream mixes made with the fresh plain condensed skimmilk whipped to 100 per cent overrun faster than ice cream mixes made with fresh superheated and fresh sweetened condensed skimmilk. There was no significant difference in the time required to reach 100 per cent overrun in mixes made with the latter two types of condensed skimmilk, but a higher maximum overrun was obtained in those mixes made with fresh sweetened condensed skimmilk. There was an increase in time required to reach 100 per cent overrun in mixes made with the three types of condensed skimmilk stored frozen one month, and the maximum overrun was lowest. The time required to reach 100 per cent overrun in ice cream mixes made with the three types of condensed skimmilk stored frozen for two and three months was less than the time required for mixes made with fresh condensed skimmilk and the maximum overrun was approximately the same as that of ice cream made with fresh condensed skimmilk.

Flavor, Body, and Texture and Melting Characteristics of the Ice Cream. In general there was no appreciable difference in the flavor scores of ice cream which could be attributed to the type of condensed skimmilk used

TABLE 3

Comparison of some chemical and physical properties of ice cream mixes, made from fresh and stored frozen condensed skimmilk

Condensed skimmilk	Ice cream mix														
Type and age	Titratable acidity			pH			Time to reach 100% overrun			Time to reach max. overrun			Maximum overrun		
	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*
Plain	%	%	%				*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	%	%	%
Fresh	.200	.188	.196	6.41	6.33	6.39	5.0	8.5	9.5	14	20	17	133	144	129
1 mo.	.200	.188	.206	6.39	6.29	6.39	7.0	11.0	8.0	14	19	13	133	132	126
2 mos.	.179	.196	.169	6.36	6.40	6.40	8.0	6.5	6.5	15	18	14	126	146	120
3 mos.	.....	.183	.183	.....	6.38	6.39	....	6.0	4.0	....	16	17	.....	136	142
Superheated															
Fresh	.200	.188	.196	6.39	6.33	6.39	6.5	9.0	9.0	15	15	18	123	134	132
1 mo.	.188	.188	.186	6.37	6.30	6.41	9.0	11.0	9.0	16	20	16	119	127	130
2 mos.	.179	.196	.173	6.23	6.40	6.36	7.5	9.0	7.0	13	14	16	124	141	125
3 mos.	.....	.178	.173	.....	6.39	6.40	....	11.0	6.0	....	20	19	.....	121	133
Sweetened															
Fresh	.200	.194	.227	6.38	6.32	6.27	7.0	9.5	8.0	14	20	20	125	144	135
1 mo.	.183	.188	.206	6.38	6.29	6.39	8.0	11.0	8.5	15	20	16	133	127	124
2 mos.	.179	.196	.183	6.36	6.40	6.40	8.0	8.5	6.5	16	16	17	133	141	127
3 mos.	.....	.183	.183	.....	6.40	6.39	....	7.5	6.0	....	17	19	.....	130	136

* Indicates date of making the condensed milk: I—October 12, 1938; II—December 29, 1938; III—February 1, 1939.

or whether it was the fresh or frozen product. From the standpoint of body and texture, there was no difference in ice creams made with the three types of fresh condensed skimmilk. However, there was a tendency for the ice creams made with the three types of condensed skimmilk stored for one month to score slightly lower in body and texture than that made with fresh condensed skimmilk.

Ice cream made with fresh superheated condensed skimmilk was slower in melting than ice cream made with either fresh plain or sweetened condensed skimmilk. Freezing and storing superheated condensed skimmilk had no consistent effect on the melting time of the ice cream. Ice cream made with fresh sweetened condensed skimmilk melted faster than ice cream made with fresh plain condensed skimmilk, but ice cream made with the same condensed skimmilk after one, two, and three months in storage showed exactly opposite results. The melting time for the ice cream made with plain condensed skimmilk stored one month did not change, whereas that of ice cream made with sweetened condensed skimmilk during the same period increased. These data indicate that freezing and storing plain condensed skimmilk brought about a change that did not occur in the sweetened condensed skimmilk. These differences, however, were not great and would not suggest an advantage of one type of condensed skimmilk over the other.

As the melting time decreased the weight of the melted ice cream also decreased. By studying the weight of the melted ice cream, the amount of foam could be relatively determined. Of the three types of condensed skimmilk used in the ice cream, the fresh sweetened condensed skimmilk gave the most foam on melting, but after the condensed had been in storage for one month, ice cream made with the plain condensed skimmilk gave the most foam. Ice cream made with superheated condensed skimmilk always gave the least amount of foam on melting. Freezing and storing the plain and superheated condensed skimmilk increased the amount of foam in the melted ice cream, whereas the opposite was true for sweetened condensed skimmilk.

Ice cream made with plain condensed skimmilk melted down smoothly and evenly in every case. The ice cream made with stored frozen superheated condensed skimmilk was frequently criticized for a slight curdy appearance, but the most noticeable difference in melting of this type of ice cream was that, instead of the sides melting straight down as they did in the ice cream made with plain and sweetened condensed skimmilk, they tended to fall off in chunks. Ice cream made with fresh sweetened condensed skimmilk always melted smoothly and evenly, but that made with stored frozen sweetened condensed skimmilk was sometimes criticised for having a flaky or curdy appearance.

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THE EFFECT OF ADDED EGG PHOSPHOLIPIDS ON THE NUTRITIVE VALUE OF CERTAIN VEGETABLE OILS[1]

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It was recently reported from this laboratory (1) that butter fat has a higher nutritive value for growth in weanling rats than certain vegetable oils when homogenized into mineralized skimmed milk and supplemented with all the known essential fat soluble vitamins. The difference in the nutritive value was not found to be due to factors contained in the non-saponifiable fraction of butter fat (1). However, since the phospholipids are decomposed upon saponification it appeared possible that the difference in the nutritive value of butter fat and the vegetable oils fed might be due to some particular phospholipid contained in the butter fat which was not contained in the vegetable oils. The work described in this paper was carried out to determine if a common available phospholipid, such as egg phosphatide, or the nitrogenous bases of phosphatides, had any effect on the nutritive value of the vegetable oils used in our experiments.

EXPERIMENTAL

Weanling rats weighing between 30 and 35 grams were used. Six rats, 3 males and 3 females, were placed on each fat in each particular experiment as described in a previous paper (1). The fats were homogenized into skimmed milk at a level of 4 per cent and fed ad libitum. Twenty micrograms of carotene were added to each gram of fat, 100 micrograms of α-tocopherol[2] were given to each rat every week and all animals were irradiated 10 minutes daily. The milks were mineralized with iron, copper, and manganese so that each 100 cc. contained 1.5 mg. of iron, 0.15 mg. of copper, and 0.15 mg. of manganese.

The phosphorus content of the oils used in these experiments was found to be approximately 10 mg. per 100 grams of butter fat, 3 mg. per 100 grams of corn oil, and 8 mg. per 100 grams of coconut oil. From the phosphorus analysis, if all the phosphorus was in the oils as phospholipids containing 4 per cent of phosphorus, the phospholipid content of butter

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should be 0.25 per cent, corn oil 0.075 per cent, and coconut oil 0.2 per cent. The phospholipid content of skimmed milk has been reported to be 0.015 per cent and of butter 0.2 per cent (2). The skimmed milk used for making up milks should have supplied about the same amount of phospholipids as the fat, but with corn oil the amount of phospholipid consumed would be considerably lower than with butter fat or coconut oil. However, soy bean oil, which was inferior to butter fat for growth in young rats (1), was found to contain as much as 120 mg. phosphorus per 100 grams, or 3 per cent phospholipids, on the basis of 4 per cent phosphorus in the phospholipids. On the basis of these results it was considered best to feed one of the common available phospholipids (egg lecithin) along with corn oil and coconut oil.

These experiments were made with egg lecithin (Pfanstiehl Pure Ex-Ovo-soluble) added to corn oil and coconut oil at a level of 0.25 per cent. Comparisons were also made with butter fat and the vegetable oils without lecithin. Some response was obtained on the oils with egg lecithin added to the oils alone but the growth was not as good as the growth obtained on butter fat. The addition of lecithin seemed to improve the appearance of the hair coat of the animals considerably.

Since some response was obtained with 0.25 per cent lecithin in the vegetable oils, a higher level was tried. In a second trial with the same experimental set up but with 0.5 per cent of egg lecithin added to the oils, no better response was obtained than was found in the first trial. A third experiment with the lecithin content of the oils at 0.5 per cent resulted in a poorer response with considerable variation. Figure 1 illustrates the

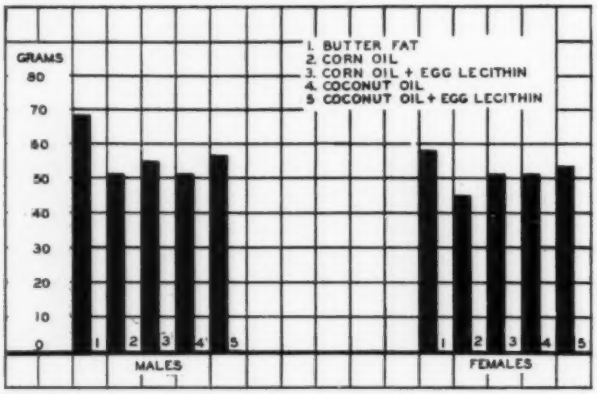

FIG. 1. The bars indicate average gains made during the first three weeks of the experiment—representing 18 rats, 9 males and 9 females, on each fat.

average weight gains made for these trials during the first three weeks on the experiment with the various oils.

Another experiment was set up comparing butter fat, corn oil, corn oil plus sphingomyelin,[3] corn oil plus sphingosine,[3] corn oil plus ethanolamine, and corn oil plus choline. The phosphatides and bases were suspended or dissolved in water and fed in the milk each day. The following amounts were fed to each rat on the particular diet per day. Sphingomyelin 0.6 mg., sphingosine sulfate 0.5 mg., ethanolamine 1 mg., and choline 1 mg. Limited sources of sphingomyelin and sphingosine sulfate prevented these substances being fed at higher levels. No particular differences were observed in weight and appearance between the animals on corn oil alone and corn oil plus the phosphatide bases except with the females on choline. However, this group of females did not do as well as the animals on butter fat. Figure 2 illustrates the average gain in weight during the first three weeks of the experiment.

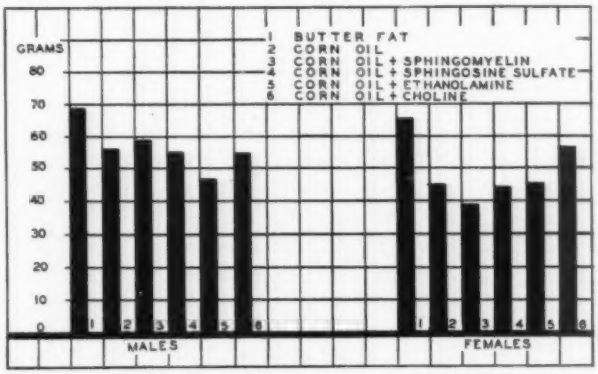

FIG. 2. Bars represent average gains made during the first three weeks of the experiment with phosphatide bases added to corn oil.

DISCUSSION

It appears from the results obtained that egg lecithin improved the nutritive value of corn oil and coconut oil slightly but not enough to give growth equal to that obtained on butter fat. In certain cases individuals on the vegetable oils plus lecithin did as well as those on butter fat but in most instances the growth was inferior to animals on butter fat. Sphingomyelin, sphingosine sulfate, and ethanolamine did not improve the nutritive value of corn oil while choline seemed to improve corn oil slightly. Since in a previous experiment (1) soy bean oil was found to be inferior

[3] We are very grateful to Dr. P. A. Levene, Rockefeller Institute for Medical Research, for the sphingosine and sphingomyelin used in these experiments.

to butter fat irrespective of its high phospholipid content, it appears that something besides the phospholipid is responsible for the superior growth obtained on butter fat with weanling rats.

CONCLUSIONS

1. Addition of 0.25 per cent and 0.5 per cent of egg lecithin to corn oil or coconut oil improved the nutritive value of these oils slightly but not enough to make them equal to butter fat when they were homogenized into mineralized skimmed milk at a level of 4 per cent and fed to weanling rats.

2. Sphingomyelin, sphingosine sulfate, and ethanolamine had no effect on the nutritive value of corn oil, but choline, in the case of the females, seemed to improve it slightly.

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THE NUTRITIVE VALUE OF THE FATTY ACID FRACTIONS OF BUTTER FAT[1]

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In a previous paper (1) it was reported that butter fat homogenized into mineralized skim milk gave better growth of weanling rats than certain vegetable oils homogenized into skim milk and fed under the same conditions with ample carotene, irradiation, α-tocopherol and minerals added in all cases. The superiority of butter fat for growth was not found to be due to factors contained in the non-saponifiable fraction of butter or to be due to compounds such as lecithin, choline, sphingomyelin or sphingosine (2). It was then thought advisable to separate the fatty acids of butter into various fractions and feed the glycerol esters of these fractions along with a vegetable oil in concentrations approximately equal to that found in butter fat.

EXPERIMENTAL

The fatty acid fractions of the butter fat were prepared as follows: Five hundred grams of melted butter fat were poured into 1000 cc. of 20 per cent alcoholic potassium hydroxide solution and heated on the steam bath for about one-half hour. The alcohol was evaporated off in an open dish, the soaps dissolved in water and the separations made similar to a procedure described by Hilditch and Jones (3). The solution was neutralized with sulfuric acid and steam distilled until about 5 liters of distillate were collected. The volatile acids were extracted from the distillate by 5 successive extractions with ether. The non-volatile fraction was washed thoroughly with hot water and dissolved in 2000 cc. of alcohol. The alcohol solution was heated almost to boiling and 200 gm. of lead acetate crystals added. The solution was allowed to cool and the insoluble lead soaps filtered off and recrystallized from alcohol. After removing, by vacuum distillation, the alcohol from the soluble lead soaps, which constitutes most of the unsaturated fatty acids, the material was washed with hot water and the free fatty acids regenerated by adding dilute sulfuric acid until the solution was distinctly acid to congo red. The acids were separated from the water and precipitated lead sulfate, washed with hot water and taken up in ether to remove the last traces of lead sulfate. The insoluble lead soaps which constitute most of the saturated acids but still contaminated

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with some of the unsaturated acids were treated in exactly the same manner as the unsaturated acids and finally taken up in ether to remove the last traces of lead sulfate. The ether was removed from the various fractions under vacuum. Five hundred gm. of butter fat yielded 36.5 gm. of volatile acids, 180 gm. of the unsaturated acid fraction and 200 gm. of the saturated fraction.

The triglycerides were synthesized as follows (4). The fatty acids from each fraction were placed in a suitable round bottom distilling flask and the theoretical amount of glycerol added as calculated from the fatty acid composition of each fraction (3). The flask containing the fatty acids and glycerol was placed in an oil bath and heated to 200° C. for six hours with a fine stream of carbon dioxide passing through the mixture. The glycerides of each fraction were then mixed with corn oil (Mazola) in the following proportions:

Triglycerides of	volatile	acids	7	parts,	corn oil	93	parts
"	" unsaturated	"	40	"	"	60	"
"	" saturated	"	55	"	"	45	"

These are approximately the proportions of the different fractions isolated from the butter.

Weanling rats about 20 days old and weighing 30 to 35 gm. were used. Six rats, 3 males and 3 females, were placed on each fraction to be tested and kept in individual cages. Five groups were set up and fed as follows, ad libitum:

Group	I.	Butter fat milk
"	II.	Corn oil milk
"	III.	Corn oil plus volatile fraction milk
"	IV.	" " " unsaturated " "
"	V.	" " " saturated " "

The fats were homogenized into fresh skim milk with a small hand homogenizer and all milks were made up to contain 4 per cent fat. Twenty micrograms of carotene were added to each gram of fat and 100 micrograms of α-tocopherol acetate[2] were given to each rat every week. All rats were irradiated 10 minutes each day. All milks were mineralized with iron, copper and manganese so that each 100 cc. of milk contained 1.5 mg. of iron, 0.15 mg. copper and 0.15 mg. of manganese.

The results obtained are illustrated in figures 1 and 2 (Experiment 17) and figures 3 and 4 (Experiment 19). The animals on corn oil plus the saturated fraction of butter fat (Group V) grew faster than the animals on butter fat and considerably faster than the animals on corn oil, or on the volatile or unsaturated fractions. The data in figures 1 and 2 do not

[2] We are indebted to Hoffmann LaRoche, Inc., Nutley, New Jersey, for generous supplies of dl-α-tocopherol acetate.

include the unsaturated fraction because shortly after mixing the triglycerides with corn oil the mixture began to thicken and develop an odor somewhat like paint. The animals on this fraction failed to grow. In the second trial, Experiment 19, figures 3 and 4, the fractions from butter were

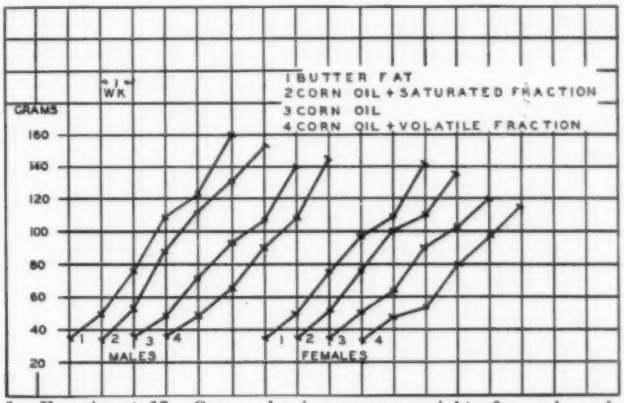

FIG. 1. Experiment 17. Curves showing average weights for each week for rats on milks made up with butter fat, corn oil and fatty acid fractions of butter fat representing 6 rats, 3 males and 3 females, on each fat.

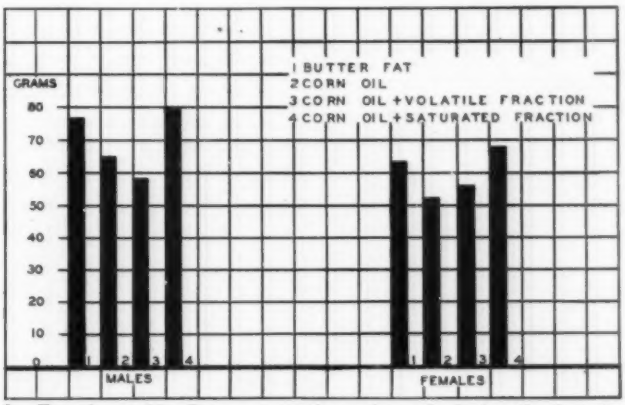

FIG. 2. Experiment 17. Bars representing gain made during the first three weeks on experiment representing 6 rats, 3 males and 3 females, on each fat.

mixed with corn oil just before being homogenized into the milk. The males on butter fat in Experiment 17, figure 2, made an average gain of 76 gm., on corn oil 65 gm., on the volatile fraction 57 gm., and on the saturated fraction 80 gm. in three weeks. The females on butter fat in this experiment made an average gain of 63 gm., on corn oil 52 gm., on the vola-

tile fraction 56 gm., and on the saturated fraction 68 gm. in 3 weeks. The males on butter fat in Experiment 19, figure 4, made an average gain of 70 gm., on corn oil 63 gm., on the volatile fraction 48 gm., on the unsaturated fraction 63 gm., and on the saturated fraction 82 gm. gain in 3 weeks. The

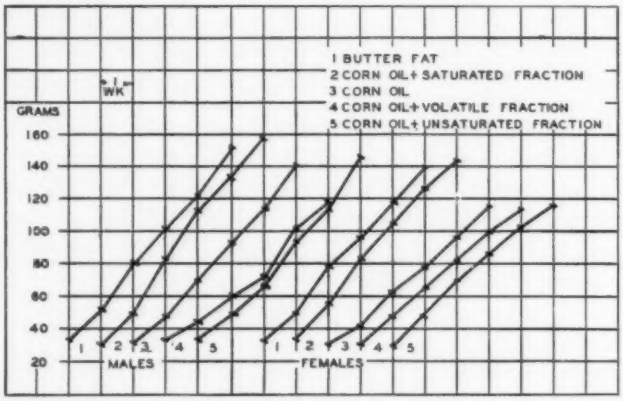

FIG. 3. Experiment 19. Curves showing average weights for each week for rats on milks made up with butter fat, corn oil, and fatty acid fractions of butter fat representing 6 rats, 3 males and 3 females, on each fat.

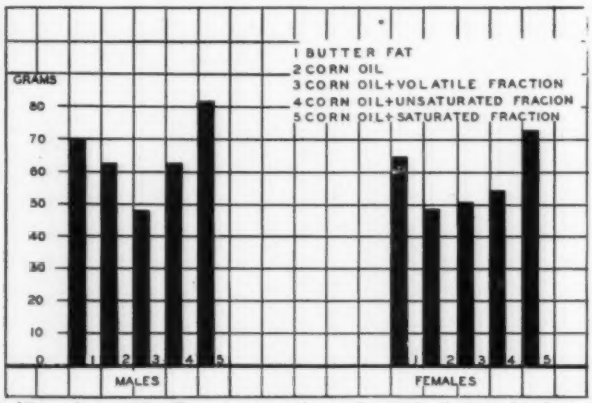

FIG. 4. Experiment 19. Bars representing gain made during the first three weeks on experiment representing 6 rats, 3 males and 3 females, on each fat.

females on butter fat gained an average of 66 gm., on corn oil 47 gm., on the volatile fraction 51 gm., on the unsatured fraction 55 gm., and on the saturated fraction 73 gm. in 3 weeks. The general appearance and condition of the fur coat of the animals on the saturated fraction was better during the first two or three weeks on the experiment than the other groups.

However, after this time there was very little difference between the groups in appearance.

Food consumption records indicate that the animals on the saturated fraction did not make more efficient gains than animals on butter. That is, growth paralleled consumption very closely. However, we have found that the faster-growing animals usually made slightly more efficient gains than animals growing at a slower rate and these differences appeared between groups of rats on butter fat and vegetable oils during the first two or three weeks of the experiment.

DISCUSSION

The results obtained indicate that the superiority of butter fat fed in the milks is due to fatty acids contained in the *saturated* fraction of butter fat. It also appears that the superiority is due to long chain saturated fatty acids. The saturated fraction was found to have an iodine number of 9.2, showing that some unsaturated fatty acids still remained. However, if the superiority of growth was due to unsaturated fatty acids then it appears that corn oil or the unsaturated fraction of butter fat should have supplied the necessary fatty acids. Hilditch and Jones (3) found the saturated fatty acid fraction of butter fats to have an iodine number of 10 and believed that the unsaturated fatty acids in the saturated fraction were only those carried over in the separation. However, no final proof can be given that an unsaturated fatty acid, being insoluble as the lead soap, is not responsible for the superiority until the saturated fraction is completely hydrogenated and fed.

No final explanation can be offered for the observation that better growth was obtained with the saturated butter fraction and corn oil as compared with butter alone. It is probable that the fatty acids which are responsible for the superiority of butter over vegetable oils are present in larger amounts in butter fat, and in the mixture of corn oil and the saturated fraction from butter fat an increased amount of saturated fatty acids of high molecular weight made possible the accelerated growth.

CONCLUSIONS

1. The fatty acids responsible for the superior growth of young rats obtained on butter fat as compared with certain vegetable oils homogenized into skim milk with all of the known essential fat soluble vitamins added, apparently lie in the saturated fraction of butter fat.

2. When the fatty acids of butter fat were separated into the volatile acids by steam distillation and into the unsaturated and saturated acids as lead soaps and the triglycerides of these fractions fed in corn oil in approximately the composition found in butter the saturated fraction with

corn oil was found to be a little superior to butter fat while the other two fractions compared favorably with corn oil.

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THE LENGTH OF THE INTESTINE OF CALVES AND ITS BEARING ON THE ABSORPTION OF THE NUTRIENTS FROM THE CHYME[1]

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In his recent book (1) Alvarez states, "Meat-eating animals have a simple stomach and colon and a short bowel, whereas grass-eating animals usually have a complicated stomach and colon, a large cecal pouch, and a long, small bowel. In carnivorous animals the small bowel is said to be only from four to eight times the body length, while in herbivorous animals it is from twenty-five to seventy-five times the body length." According to Swett and Graves (15) the length of the small intestine of mature beef cows (eight animals) varies from 93 to 140 feet and that of dairy cows (two animals) from 144 to 172 feet. From these data one finds that the post-mortem length of the small intestine of beef and dairy cattle is 28 and 33 times, respectively, the length from withers to pin bones, or within the lower limit suggested by Alvarez. The length of the large intestine of the same animals varies from 23 to 41 feet for beef animals and from 43 to 46 feet for dairy animals.

Black, Semple and Lush (2) found that the average length of the small intestine of 20 seven-months old range steers was 98 feet. One hundred and twenty days later the average length of the small intestine from a similarly bred group (32 animals) had increased to 110 feet. The average length of the large intestine for both groups was 21 feet. Such figures, of course, are only approximate in that the changing tone of the musculature of the intestine makes an exact figure impossible.

Data concerning the length of the intestine of a live calf has only been reported on one animal (6). In this instance the length of the small intestine of a six months old calf was found to be 21 feet two inches in the living animal and 68 feet nine inches on removal from the body. Since that time the following data shown in table 1 have been accumulated:

From these data it is apparent that the length of the small intestine of the living calf is about seven times its body length, a figure somewhat out of line with that suggested by Alvarez.

What influence this greater length of the small bowel has on digestion can be better understood by studying the movement of chmye in the gastrointestinal tracts of carnivora and herbivora. Heile (10) states that in dogs on a mixed meal, material began to appear at the lower end of the

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TABLE 1

Length of small and large intestinae of calves in vivo and post mortem

Calf number	Age in mo.	Length of small intestine				Length of large intestine			
		In vivo		Post mortem		In vivo		Post mortem	
		Ft.	In.	Ft.	In.	Ft.	In.	Ft.	In.
47A4	5	23	3	77	1	....	....	....	....
47R	9	20	1	53	4	7	6*	12	10*
1698	12	17	2	68	10	7	1	14	8
1704	10	25	5	81	1	7	2	16	10
Previously reported (6)	6	21	1	68	9	....	....	....	....
Average		21	5	69	10	7	2	15	9

* Data do not include cecum. Consequently figures have not been used in calculating average.

small bowel one hour after feeding and by the fifth hour the small bowel was empty. With a diet rich in carbohydrates and fats, the time required for digestion and elimination from the stomach and small intestine was only three to four hours. Assuming the usual time for digestion in the stomach of dogs, it is apparent that the absorption of the digestible portion of the meal and the elimination of the residue into the large gut occurs within an hour after the food enters the small intestine. In other words, if the average length of the small bowel is about four feet (1, 3) the chyme must be moving through this part of the intestinal tract at an average rate of at least four feet per hour.

Comparable data for ruminants are not available. Dickey (4) found that the first part of a meal of milk passed through the entire gastrointestinal tract of young calves in 30 hours while Fish (8) reported that the first of a dye marked feed offered to mature cattle passed through the gastrointestinal tract in 16 to 17 hours. The time required for the passage of all the food residues from one meal through the digestive tract of herbivora may require several days (5). Ewing and Smith (7) claim that during most of this time the food is in the rumen. It is entirely probable that the first residues to reach the anus from any one meal are from food which has not been detained long in any part of the stomach. The average time for the major portion of the food to pass through the gastrointestinal tract of mature herbivora probably is about 72 to 84 hours, depending on the amount and type of feed, its physical condition, and certain physiological reactions in the animals (7). What then is the rate at which the food residues pass through the various segments of the intestine?

Available information would indicate that food nutrients other than the fiber fraction are equally well absorbed by ruminants and non-ruminants despite a difference in the length of their respective intestinal tracts. Due to the physical effect of the fiber the progress of food through the small

intestine of herbivora is probably faster than for carnivora. In fact, this would seem logical from two unrelated bits of evidence. First, Krzywanek (12) and others (9, 11, 16) found that the rate of travel of the ingesta is speeded up by giving food at frequent intervals. The act of filling the stomach also increases the motility of the colon, frequently to the point of causing immediate defecation. With ruminants it is a well known fact that food from the first two compartments of the stomach is emptied into the abomasum at frequent intervals. This frequent stimulus may aid in the more rapid progress of the chyme through the bowel of the ruminant as compared to that of the non-ruminant.

A factor of even more importance in increasing the rate of passage of food through the bowel is the fiber content. Foods rich in cellulose tend to pass through the small bowel faster than low fiber feeds (13, 14). This in itself may explain why a ruminant needs a longer small intestine, relatively, than a dog in order to utilize its feed efficiently.

It has been the experience of the investigators that high fiber material, as used in most "milk substitutes," tends to cause young calves to scour. Unground or coarsely cut roughage does not have this effect, probably because it is held in the rumen until partially "digested." This predisposition to scour on ground high fiber feeds vanishes as soon as the first three compartments of the stomach have reached sufficient size to hold back one or two days' food supply until acted upon by bacteria and the rumen fluid. Apparently, the intestine of the young calf is quite sensitive to large amounts of fiber. Although the small intestine of the ruminant is two to three times as long, relatively, as that of the non-ruminant, this additional length is not sufficient to provide for the efficient use of high fiber feeds unless thoroughly comminuted in the rumen and passed into the abomasum in small amounts. Grinding of the feed cannot replace this action of the rumen.

The large intestine of the ruminant is relatively short. In that its length is comparable to that of the large bowel in carnivora, there is no reason for believing that it plays an active part in the utilization of a high fiber feed.

SUMMARY

The small intestine of the living calf is about seven times the body length, or about one-third the post-mortem length. Variations in the ratio between body length and length of intestine depend more on individuality than upon the age of the calf. Although the progress of the chyme through this region is comparatively rapid, the increased length in ruminants allows for proper absorption of the nutrients if thoroughly comminuted in the rumen.

The large intestine does not show as great a difference in length between the living and post-mortem stages as does the small intestine.

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EFFECT OF SALT ON THE KEEPING QUALITY OF CREAM[1]

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INTRODUCTION

The problem of preventing deterioration in cream from the time it is produced until it is finally made into butter is of vital concern to the creamery industry. In many cases the limited volume of cream produced per farm may not justify the expense involved in providing adequate facilities for cooling and storage; and daily or even frequent deliveries of the cream cannot be expected. Before delivery to the cream station or creamery, much of the cream used in the manufacture of butter has been held for several days at temperatures favorable for rapid growth of the microorganisms which cause deterioration.

The greatest need in improving the quality of cream and butter produced throughout much of the Middlewest is in finding some inexpensive, simple procedure which could be used by the producer to prevent or retard cream deterioration. Williams (1) of the United States Department of Agriculture patented a method of preserving cream by the addition of salt. This patent (U. S. patent No. 2,192,864 granted in January, 1939) has been dedicated to the free use of the people of the United States. The use of this method, however, has not been legalized by the United States Food and Drug Administration, which contends that cream to which salt has been added is an adulterated food product.

This investigation was undertaken to determine the merits and the limitations of improving the keeping quality of cream by the addition of salt. The principal objectives of this study were:

1. To determine the effectiveness of various concentrations of salt in retarding or preventing deterioration of sweet cream stored at various temperatures.
2. To determine the effectiveness of salt in stopping deterioration in cream stored at 70° F. for various intervals before the salt was added.
3. To determine the effect of salt in cream on the quality of the resulting butter.
4. To determine the effect of salt in cream on the accuracy of the Babcock test.

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REVIEW OF LITERATURE

Williams (1) claimed that cream to which about seven per cent salt had been added could be held for one week at room temperatures without appreciable deterioration, and such cream could be used in the manufacture of sweet cream butter; or if reseparated into a plastic cream, it could be used as a substitute for sweet market cream.

Thompson and Macy (2) indicated that the addition of 7.5 to 10 per cent salt to cream stored for 10 days at temperatures of 70° F. or lower, effectively retarded bacterial growth and acid development, and prevented the cream from developing off-flavors except for a slight staleness. Control lots of the same cream held without salt were criticized for such defects as "cheesy," "yeasty" or "alcoholic."

Castell and Garrard (3) reported that sweet fresh cream containing 7 per cent salt could be held in a satisfactory condition for eight days at 77° F. They observed that oxidizing bacteria, believed to be responsible for cheesy and rancid flavors, were almost completely inhibited in the salted cream. They also found that butter produced from salted cream stored for eight days at 60 to 77° F. was superior to butter made from unsalted cream stored at 50° F. for eight days.

The data which have been published appear entirely favorable to the use of salt for improving the keeping quality of cream. Further studies using more samples of cream and a wide range of storage temperatures seemed desirable since lower storage temperatures than those which generally prevail throughout the Middlewest were used in previous studies.

METHODS

Four series of experiments were conducted to determine the possible relationships between salt concentration, time and temperature of storage, and keeping quality of cream. In each series, which consisted of 20 samples, salt was added to 30 per cent cream in amounts equivalent to 0, 7, 10, 13 and 16 per cent of the weight of the fat-free serum and samples of the cream were held at 60, 70, 82 and 90° F., respectively, for 10-day periods. Changes in grade, acidity, and formol titration were followed, observations being made at 1, 2, 3, 4, 5, 6, 8, and 10 day periods. Changes in the bacterial flora of the 40 samples of cream in trials III and IV were followed by direct microscopic observations at the same intervals.

Samples for grading were labeled to conceal their identity, thereby eliminating much of the inevitable personal factor when organoleptic methods are employed. After each sample had been graded independently by two persons, the indicated grade was determined by mutual agreement. The grade designations used were: sweet, first, second, and third grades. The definitions for cream grades as given in the Kansas dairy law were used as a basis for grading (4). In the grading of salted cream samples, saltiness was not considered as an undesirable flavor defect.

Four trials consisting of 24 samples were conducted to determine the effectiveness of salt in preventing deterioration in cream held at 70° F. for various intervals before the salt was added. In each trial, a lot of fresh 30 per cent cream was divided into six one-pint samples. One sample was held without salt throughout the storage period, salt in 13 per cent concentration (serum basis) was added to a second sample immediately, while the remaining samples were held for 3, 4, 5, and 6 days respectively before salt in the same concentration was added. Changes in grade, acidity, and formol titrations were observed throughout the remainder of a seven-day storage period.

Three preliminary trials were conducted to determine the effect of salt in cream on the quality of the resulting butter. In each of these trials, salted cream (13 per cent salt serum basis) and control lots of the same cream without salt were held in sterile glass containers for 10 days at 70° F. The samples of cream were then neutralized if necessary, pasteurized at 150° F. for 30 minutes and churned, unsalted butter being made. The resulting butter was scored fresh and again after 60 days' storage at 0 to −10° F.

When cream to which salt had been added was tested for butterfat by the usual Babcock test procedure, two principal difficulties were encountered: (1) The reaction between the sulphuric acid and salted cream frequently caused part of the sample to boil out over the top of the test bottle, and (2) a grayish brown deposit of foreign material, which interfered with the reading of the tests, frequently occurred at the base of the fat column.

To overcome these difficulties the conventional Babcock test procedure was modified in the following respects: (1) An equal volume of cold water was added to the cream sample in the test bottle before the acid was added, and (2) the acid (17.5 ml.) was added in three equal portions with a delay of 10 minutes between the first and second additions of the acid and with a delay of five minutes between the second and third additions of the acid.

Using this modified Babcock test procedure 50 individual tests were performed on samples of salted cream by each of two operators, and the results compared with the calculated test of the samples. The calculated value was arrived at by running not less than 12 replicate tests on the unsalted cream and then calculating the theoretical test of the sample after salt was added.

Quantitative and qualitative changes in the microflora of cream were followed by direct microscopic observations (6). The method ordinarily employed for milk was used except that dilutions of one part of cream in nine of water were prepared from those samples in which the count had become so high as to make enumeration of microorganisms difficult. Observations were made only on samples of cream in series III and IV.

RESULTS

Effectiveness of salt added to cream immediately after separation in preventing deterioration of sweet cream held at a series of different storage temperatures. The effectiveness of salt in retarding deterioration in sweet cream was found to be directly related to the concentration of salt used and the time and temperature of storage. The data are presented in table 1.

TABLE 1

Change in grade of cream as affected by salt concentration and storage temperature

Sample No.	Per cent salt (serum basis)	Grade of cream after 5 days storage (sweet, 1, 2, 3)				Grade of cream after 10 days storage (sweet, 1, 2, 3)			
		Lot I	Lot II	Lot III	Lot IV	Lot I	Lot II	Lot III	Lot IV
				Cream held at 60° F.					
1a	0	2–	2–	1	1–	3	3	1–	1–
2a	7	sw	sw	sw	sw	1	1	1	1
3a	10	sw	sw	sw	sw	sw–	sw	sw	sw
4a	13	sw	sw	sw	sw	sw	sw	sw	sw
5a	16	sw	sw	sw	sw	sw	sw	sw	sw
				Cream held at 70° F.					
1b	0	2–	2–	1–	1–	3	3	2	3
2b	7	1	1	1	1–	1	1	1	2
3b	10	sw	sw	sw	sw	sw–	sw–	sw	1
4b	13	sw	sw	sw	sw	sw	sw	sw	1
5b	16	sw	sw	sw	sw	sw	sw	sw	sw
				Cream held at 82° F.					
1c	0	3	3	2–	3	3	3	3	3
2c	7	1	1–	1–	2	2–	2–	1–	2
3c	10	sw–	1	sw	1	1	2–	2	2
4c	13	sw	1	sw	sw	1	1	1–	1–
5c	16	sw	sw	sw	sw	1–	1	sw	sw
				Cream held at 90° F.					
1d	0	3	3	3	3	3	3	3	3
2d	7	1–	2	2	2	2–	2–	2	2–
3d	10	1–	1	1	1–	2–	2–	2	2
4d	13	1	sw–	sw	sw–	2	2–	2	1–
5d	16	sw	sw	sw	sw	1–	1	1	2

Control lots of cream to which no salt had been added showed rapid deterioration, the rate and extent of deterioration (measured by organoleptic grading) being most rapid at the higher storage temperatures. This is indicated by the data presented in table 1 which shows that all of the control samples became unlawful within five days at 82 and 90° F. with the exception of one sample, whereas when samples of the same cream were held at 60 and 70° F., none of the cream was unlawful and four of the eight samples were still first grade at the end of a similar storage period. After 10 days' storage all of the control samples held at 70, 82

and 90° F. were unlawful with but one exception, whereas only two of the four samples held at 60° F. were unlawful, the two remaining samples being poor first grade cream.

The addition of seven per cent salt (serum basis) to the cream held at 60° F. kept each of the four samples of cream sweet without exception for five days and from going below first grade during a 10 day storage period. When the storage temperature was increased to 70° F., all four samples containing seven per cent salt were first grade after five days and with but one exception after a 10-day storage period. This concentration of salt, however, did not prevent some of the cream from becoming second grade during a five day storage period at 82 or 90° F. One of the four samples stored at 82° F. and three of the four samples stored at 90° F. were second grade after a five day storage period. After 10 days' storage at these temperatures all of the samples with but one exception were second grade or lower.

Salt in 10 per cent concentration kept all of the cream sweet at 60 and 70° F. for a five-day period and for 10 days at 60° F. This concentration of salt prevented any of the samples from becoming second grade when held for five days at 82 and 90° F., or for 10 days at 70° F. When stored at 82 and 90° F. for 10 days only, one of the eight samples containing 10 per cent salt was first grade, the remaining samples being second grade.

Cream containing 13 per cent salt remained sweet for 10 days at 60 and 70° F. with the exception of one sample which was still first grade cream and for five days at 82 and 90° F. with the exception of one sample each, neither of which had dropped below first grade. After 10 days' storage at 82° F. two of the four samples were graded as first grade cream and the remaining two samples as poor first grade cream. After10 days' storage at 90° F. only one of the four samples was above second grade.

All samples of cream containing 16 per cent salt (serum basis) remained sweet during five days' storage at each temperature used and during 10 days' storage at 60 and 70° F. This salt concentration was effective in keeping all samples stored at 82 and 90° F. from becoming second grade with the exception of one sample during the 10-day storage period.

Acidity. The results of the acidity test tend to corroborate the organoleptic grading. The rate and extent of acid development which occurred during the 10-day storage period was found to be dependent upon the salt concentration, the initial quality of the cream, and the time and temperature of storage (figure 1).

The rate of acid development was rapid during the first few days of storage in all of the control lots of cream to which no salt had been added, and was greatly accelerated at the higher storage temperatures.

Salt in 7 per cent concentration prevented any marked increase in

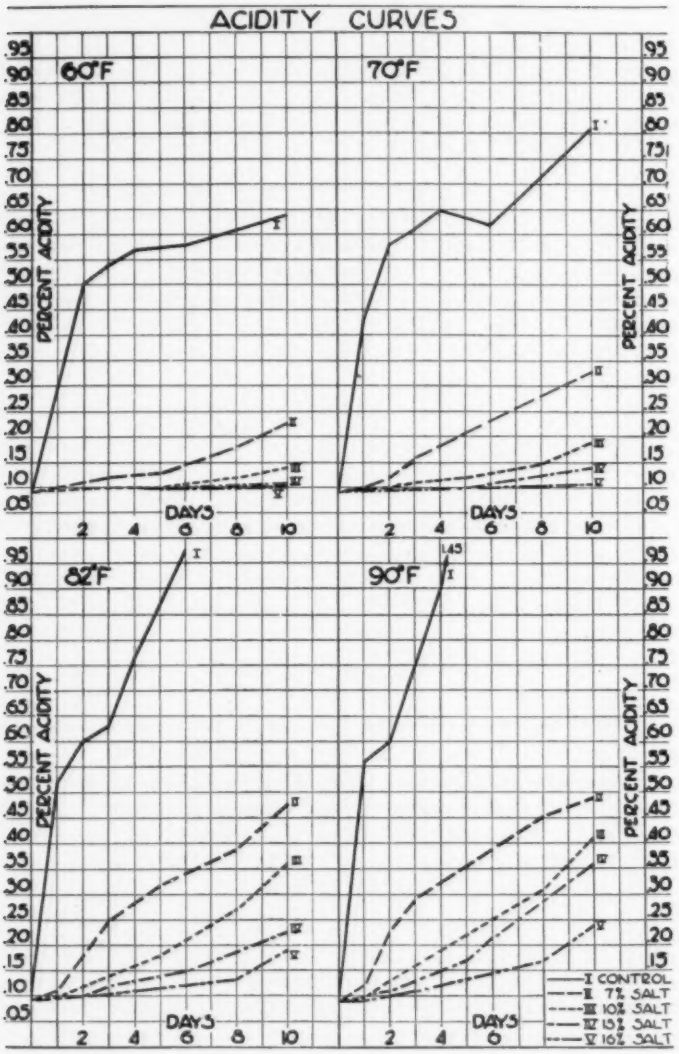

FIG. 1. Effect of salt concentration and temperature of storage on the titratable acidity of cream.

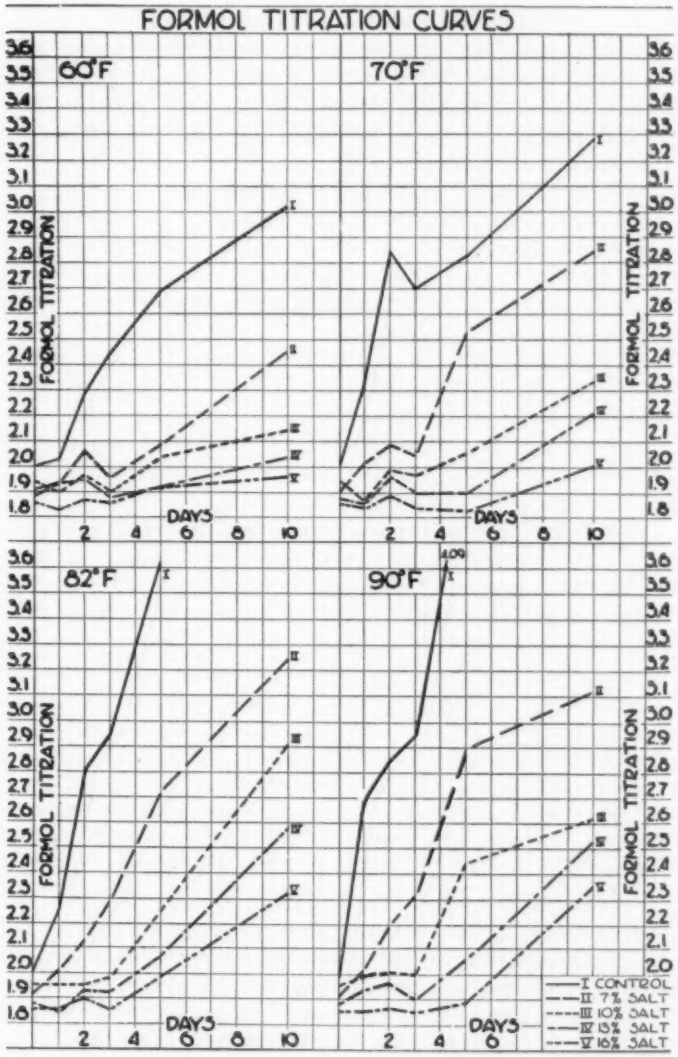

FIG. 2. Effect of salt concentration and temperature of storage on the formol titration of cream.

acidity at 60° F. throughout the 10-day storage period and greatly retarded acid development in samples stored at higher temperatures.

When 10 per cent salt was used no appreciable increase in acidity was observed after five days' storage at 60 to 70° F. and after 10 days the average acidity did not exceed 0.20 per cent at these temperatures. At 82 and 90° F., however, 10 per cent salt did not prevent the cream from becoming sour after 10 days.

Thirteen per cent salt prevented all of the samples stored at 60 or 70° F. from exceeding 0.15 per cent acid during a 10-day period and was effective for five days at 82 and 90° F. in preventing the acidity from increasing above 0.17 per cent. After 10 days the average acidity was 0.23 per cent for the samples stored at 82° F. and 0.36 per cent for the samples stored at 90° F.

When 16 per cent salt was used no appreciable increase in acidity occurred in the samples stored at 60 or 70° F. for 10 days. Although there was some increase in acidity at 82 and 90° F., the maximum acidity observed was 0.24 per cent, indicating the effectiveness of this salt concentration in retarding acid development at all of the storage temperatures.

Formol titration. The effectiveness of salt in preventing proteolytic decomposition of the cream as measured by the formol titrations (figure 2) reflect the same general trends that were shown by changes in acidity (figure 1). The data show that proteolytic decomposition was more rapid in the control samples and that the rate and amount of decomposition was accelerated by an increase in the storage temperatures.

Addition of seven per cent salt did not prevent an appreciable increase in the formol titration values during a 10-day holding period at any of the temperatures used. Ten per cent or more of salt was effective at 60 and 70° F. in preventing any marked increase in the formol titration values during 10 days, but was considerably less effective at 82 and 90° F. Thirteen per cent salt prevented any marked change in the formol titration during five days at all temperatures used, but did not prevent a considerable increase after 10 days at 82 and 90° F. When 16 per cent salt was used no increase in the formol titration value of the cream was observed during five days at all temperatures or after 10 days at 60 and 70° F. However, the formol titration values increased from 1.86 to 2.33 at 82° F. and from 1.86 to 2.36 at 90° F. during the storage period. Data indicate that none of the concentrations of salt used were entirely effective in preventing increases in the formol titration values at the higher storage temperatures.

Bacteriological. The changes in the numbers of bacteria in the various lots of cream in series III and IV are shown in figures 3 and 4. In all cases the counts of the control samples reached levels of a billion or more, the time required to reach this level being longer at the lower temperatures.

At 82 and 90° F. the flora changed from cocci to rods as the holding period increased toward the time when the cream became second or third grade. In some instances appreciable numbers of yeast were found as would be expected from the development of yeasty flavors in some samples.

The addition of salt markedly retarded the development of bacteria, the greater the amount of salt the greater being the inhibitory action. In the presence of the salt the numbers of bacteria were never found to reach the large numbers which were characteristic of the maxima of the control

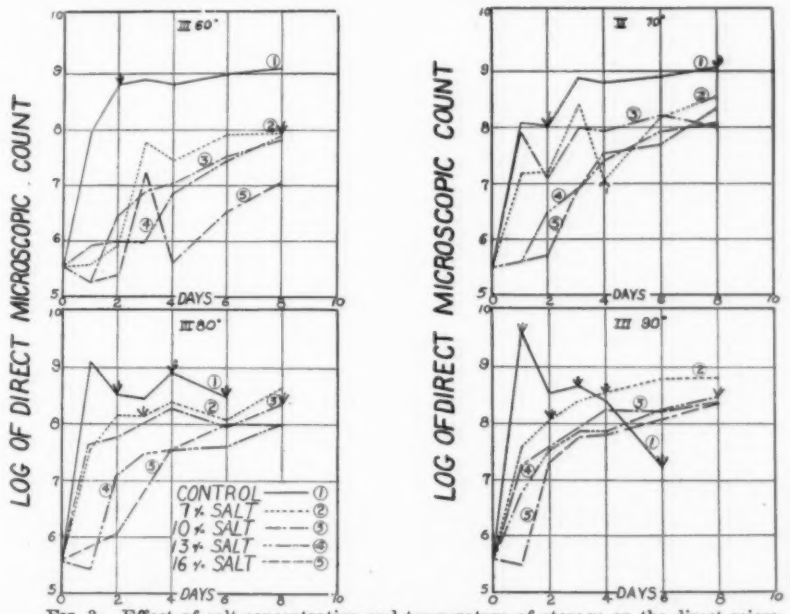

FIG. 3. Effect of salt concentration and temperature of storage on the direct microscopic count of cream samples of Lot III. (' indicates change of grade.)

samples. In the samples containing salt the flora was largely micrococci, especially in those containing the larger amounts of salt. A few short-chain streptococci, possibly enterococci, were found in the samples containing 7 and 10 per cent salt. Not only does the added salt inhibit the growth of bacteria; it also influences the types of organisms which develop. Both of these factors undoubtedly are responsible for the markedly improved keeping quality of cream to which salt in appropriate quantity has been added.

Effectiveness of salt in stopping deterioration of cream. The addition of 13 per cent salt on the serum basis to cream held at 70° F. for three or more days before the salt was added did not prevent further deterioration

of the cream as measured by organoleptic grading of the samples (table 2). The samples to which the salt was added after three or more days of storage generally graded as low or lower than the corresponding control lots of cream to which no salt had been added. On the other hand, the cream

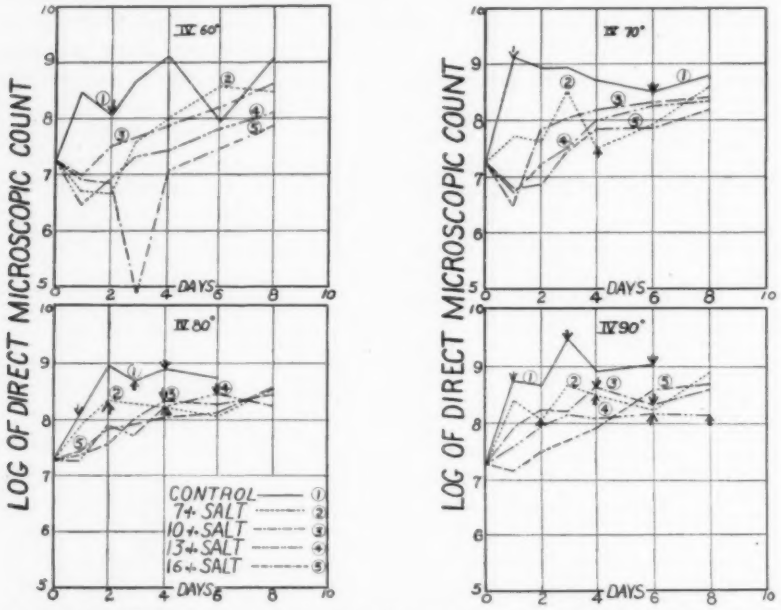

FIG. 4. Effect of salt concentration and temperature of storage on the direct microscopic count of cream samples of Lot IV. ([1] indicates change of grade.)

TABLE 2

Change in grade of cream as affected by adding salt at varying intervals of time during storage at 70° F.

Time when salt was added	Per cent salt added (serum basis)	Grade of cream after 7 days storage			
		Lot I	Lot II	Lot III	Lot IV
Check (no salt)	0.0	2	1–	2	2–
Immediately	13.0	sw	sw	sw	sw
After 3 days	13.0	1–	1–	2	3
After 4 days	13.0	1–	2	2	2–
After 5 days	13.0	2	2	2–	3
After 6 days	13.0	2–	1–	2	3

to which the salt was added immediately after separation remained sweet in each of the trials. The data indicate that the salt must be added to the cream soon after separation to be effective in preventing deterioration.

Effect of salt preservation of cream on quality of butter. Butter churned from cream to which 13 per cent salt on a serum basis had been added before holding for 10 days at 70° F. scored from two to five points higher than did the butter produced from control lots of the same cream held without salt (table 3). The butter churned from the salted creams scored 92, 92,

TABLE 3

Effect of salt in cream on the score of the resulting butter
(Cream stored at 70° F. for 10 days)

Lot number	Butter score—freshly churned			Butter score—after storage*		
	Salted cream†	Unsalted cream	Difference	Salted cream†	Unsalted cream	Difference
1	92.0	87	5.0	92.0	87	5.0
2	92.0	90	2.0	92.0	90	2.0
3	92.5	90	2.5	92.5	90	2.5
Average	92.2	89	3.2	92.2	89	3.2

* Butter stored for 60 days at 0° F. to – 10° F.
† Thirteen per cent salt added, serum basis.

and 92.5, respectively, whereas the control samples scored 87, 90, and 90, respectively. There was no change in score of any of the samples after a 60-day storage period of 0 to – 10° F., indicating that there was no appreciable tendency for chemical change in the butter produced from the salted cream. The samples of butter were analyzed for salt and in no instance was the salt content in excess of 2.5 per cent.

Although the data presented represent only three lots of cream, they do indicate that considerable improvement in the quality of butter churned from cream held without adequate cooling for considerable periods of time can be obtained by the addition of a suitable quantity of salt to the cream.

Effect of salt in cream on the accuracy of the Babcock test. The results of the modified Babcock test on salted cream are shown in table 4. Nearly half of the tests (45 per cent) checked within 0.2 per cent of the calculated test, and approximately 80 per cent of the tests checked within 0.5 per cent of the calculated test. In only two of the 100 tests did the results vary as much as 1.0 per cent or more from the calculated test. The variations between 50 individual tests of salted cream by each of two operators and the calculated fat percentages were no greater for one operator than for the other. Seventy-six per cent of the tests by operator No. 1 and 82 per cent of the tests by operator No. 2 were within 0.5 point of the calculated test.

The data indicate that salted cream samples may be tested with about the same degree of accuracy as is now attained with the conventional Babcock test procedure for unsalted cream. The modified test proposed

TABLE 4

Variations between the actual and calculated fat percentages of salted cream

Variation from the calculated test in points	Operator 1	Operator 2	Total No. of tests	Per cent of tests	Cumulative per cent of tests
	No. of tests	No. of tests			
0.0	2	8	10	10	10
0.1	17	10	27	27	37
0.2	6	2	8	8	45
0.3	4	14	18	18	63
0.4	7	1	8	8	71
0.5	2	6	8	8	79
0.6	2	4	6	6	85
0.7	8	0	8	8	93
0.8	1	4	5	5	98
0.9	0	0	0	0	98
1.0	0	1	1	1	99
1.1	1	0	1	1	100
Total	50	50	100	100	—

Note: A modified Babcock test procedure was used in testing the salted cream samples.

for salted cream does have the disadvantage of being more time-consuming than the conventional Babcock test for unsalted cream.

CONCLUSIONS

1. The results obtained in this study indicate that deterioration in cream held in glass containers as measured by organoleptic grading, acid development, formol titration, and direct microscopic examination may be definitely retarded by the addition of salt immediately after separation.

2. The amount of salt necessary to effectively prevent deterioration in cream was found to be dependent upon the time and temperature of storage. The results indicate that not less than 10 per cent salt (serum basis) would be required to prevent cream from becoming second grade when held for 10 days at 60 or 70° F. or for five days at 82 and 90° F. Since most cream is delivered within five days after it is produced, the addition of not less than 10 per cent salt (serum basis) should be adequate to enable the producer to market either sweet or first grade cream.

3. The addition of 13 per cent salt (serum basis) to cream held at 70° F. for three or more days before the salt was added did not prevent further deterioration of the cream. Thus the method is limited largely to farm use.

The above results were obtained by holding the cream in glass containers. In their interpretation consideration should, therefore, be given to the possible effects of prolonged exposure in the metallic cream container, to salted cream and to the corrosive action of the brine on the can, which may shorten the life of the can and in addition may cause metallic action damaging to the flavor and keeping quality of the cream and of the resulting butter.

4. Butter churned from cream to which 13 per cent salt (serum basis) was added at the beginning of a 10-day storage period at 70° F. scored two to five points higher than did butter produced from control lots of the same cream held under similar conditions without salt. In no case did the salt content of the butter churned from cream to which 13 per cent salt had been added exceed 2.5 per cent.

5. When a modified Babcock test procedure was used, results which agreed favorably with the calculated butterfat percentages were obtained.

6. Data obtained in this study as well as studies by other investigators indicate that the preservation of cream by the addition of salt has definite merit. Since salt is used in the preservation of many other foods and is a normal constituent of butter as usually marketed, the condemning of cream which contains added salt does not seem to be justified.

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THE SPECIFICITY OF THE LACTOGENIC HORMONE IN THE INITIATION OF LACTATION[1]

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In the recent literature Folley and Young (1, 2) have raised two questions in regard to the relation of the physiological activity of the "lactogenic" factor (as assayed with pigeons) and other hormones of the anterior pituitary in stimulating the lactation process. The first question was whether the factor stimulating the crop gland of the pigeon was identical with the factor which stimulates lactation in mammals and the second question was whether there were not several factors in the anterior pituitary which possessed lactogenic properties. It was suggested that the glycotropic factor has this property. They concluded that *"those extracts which stimulate the secretion of normal milk in the mammal should be described as containing a lactogenic substance or substances."*

The writers (3) recently presented data indicating that lactogenic preparations as assayed with pigeons and with rabbits gave comparable potency, and evidence was presented which indicated that lactogenesis and pigeon crop gland proliferation were due to the same factor. A similar conclusion was reached by Lyons (4) in comparison of pigeon crop gland proliferation and lactation stimulation in estrus guinea pigs.

The second question raised represents a difference of opinion as to the definition of a "lactogenic" hormone. We believe that the term *"lactogenic"* should be reserved for hormones which will initiate lactation in non-lactating mammary glands of animals when conditioned for lactation (by previous pseudo-pregnancy, estrus, estrogen and progestin or the mammogens). In other words, the hormone should stimulate lactogenesis rather than augment established lactation.

The object of the present paper is to present data indicating that if the term "lactogenic" hormone is restricted to anterior pituitary extracts which will initiate lactation in animals with glands conditioned to lactate, then there is only one lactogenic hormone and that fraction proliferates the pigeon crop gland.

EXPERIMENTAL

In a previous paper (5) a method of separating an initial anterior pituitary extract into two fractions, one rich in the lactogenic hormone and the

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other containing the thyrotropic and other hormones was described. The extracts used were prepared from cattle pituitaries.

The "lactogenic hormone" fraction was assayed by the McShan-Turner (6) method and contained 5000 pigeon units per gram. Only traces of the other pituitary hormones were present (7). The "thyrotropic and other hormone" fraction was rich in the thyrotropic hormone and contained 4000 Bergman-Turner (8) guinea pig units per gram. It also contained 638 Bergman-Turner (9) guinea pig units of carbohydrate metabolism hormone, 2500 Bergman-Houchin-Turner (7) chick units of gonadotropic hormone, 100 McShan-Turner (6) pigeon units of lactogenic hormone and 16.7 Houchin-Turner (10) guinea pig units of fat metabolism hormone per gram of extract.

It seemed of interest to determine the ability of these two extracts to initiate lactation in pseudo-pregnant rabbits. If the thyrotropic fraction would also initiate lactation in such animals, yet was practically free of pigeon crop gland stimulating potency, it would furnish good evidence of the individuality of lactogenesis in contrast to crop gland effect and the presence of at least two lactogenic hormones.

The extracts were all injected at their iso-electric point. The dried powder was dissolved in a slightly alkaline solution with NaOH, then precipitated by bringing the solutions to their iso-electric point with HCl. The concentration of the fluid was 1 per cent (1 cc. = 10 mg.).

Lactogenesis with "Lactogenic Hormone" Fraction. The lactogenic hormone fraction was assayed for its ability to initiate lactation in the pseudo-pregnant rabbit according to the Gardner-Turner (11) method. When injected at the rate of 1 mg. per 100 gms. body weight the glands were mostly (+) to (++), the average rating being 1.67; when 1.5 mg. was used (+++) to (++++) glands were obtained, the average rating being 3.17, which is considered the unit response (3). At this level approximately 52 mg. containing 260 McShan-Turner pigeon units in rabbits weighing an average of 3400 gms. was required. It thus appeared that about 1.5 mg. of the lactogenic hormone per 100 gm. body weight would initiate copious lactation in rabbits.

Lactogenesis with the "Thyrotropic and Other Hormones" Fraction. With the potency of the "lactogenic hormone" established for lactogenesis in rabbits, the next step was to determine whether equivalent dosages of the thyrotropic fraction would initiate lactation in pseudo-pregnant rabbits. Five rabbits were injected at the rate of 2 mg. per 100 gms. body weight. One animal died the second day. The other 4 animals showed no signs of lactation, not even duct lactation, which occurs in a low percentage of pseudo-pregnant rabbits. From these results it is clear that lactation was not initiated by amounts of the thyrotropic extract which were sufficient, in the case of lactogenic fraction, to stimulate maximum distension of the glands with milk (table 1).

Eleven rabbits were then injected with the thyrotropic fraction at the rate of 5 mg. per 100 gms. body weight. Several of these animals died after a single injection. Six animals survived the test period and of these four showed no signs of lactation. Only a single animal showed some evidence of stimulation (++) beyond that occasionally seen in pseudo-pregnant animals (table 1).

The cause of death of a considerable number of the animals is believed to be due to the large amount of the thyrotropic hormone which the extract contained. The animals appeared especially susceptible when they were kept in a warm room or during warm days in the spring. It is rather surprising, however, that the action would be so rapid; a number of animals died following the first injection.

The third group of rabbits was injected at the rate of 10 mg. per 100 gms. body weight. Special precautions were taken to keep the animals outside and at a relatively low temperature. Of six animals injected at this level, only one showed any signs of lactation. An explanation for the rather good lactation observed in this one case cannot be advanced. It is

TABLE 1

Lactation response in normal pseudo-pregnant rabbits with the "thyrotropic and other hormones" fraction

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman-Turner thyrotropic units (guinea pigs)	McShan-Turner lactogenic units	Gardner-Turner rating of lactation
	gms.	*mg.*	*mg.*			
1	3050	2	62	248	6.2	0
2	2800	2	.....	.....	.....	Died 2nd day
3	4000	2	80	320	8.0	0
4	3840	2	76	304	7.6	0
5	2760	2	56	224	5.6	0
1	3550	5	180	720	18	+ Died 6th day
2	3430	5	170	680	17	++
3	3920	5	.....	.....	.....	Died 1st day
4	2790	5	140	560	14	0
5	3650	5	185	740	18.5	0 to +
6	3680	5	.....	.....	.....	Died 1st day
7	3430	5	.....	.....	.....	Died 1st day
8	3830	5	190	760	19.0	0
9	3740	5	185	740	18.5	0
10	3970	5	200	800	20.0	0
11	3210	5	.....	.....	.....	Died 2nd day
1	3080	10	300	1200	30	+++
2	3560	10	360	1440	36	0
3	3470	10	350	1400	35	0 to +
4	3280	10	330	1320	33	0
5	3810	10	380	1520	38	0
6	3410	10	340	1360	34	0 to +
1	2950	20	400	1600	40	++ Died 4th day

possible that a few rabbits are responsive to much less lactogenic hormone than is usually required.

Finally, a single rabbit was injected with 20 mg. per 100 gms. body weight. Although this animal died on the 4th day of injection, it showed signs of lactation.

From this series of experiments, it seemed reasonable to draw the conclusion that the "thyrotropic and other hormones" fraction of the anterior pituitary does not contain the factor for lactogenesis when injected in amounts five to ten times as great in weight as the requirement of the "lactogenic" fraction to induce maximum lactation. In the maximum dosages, the units of the "lactogenic" hormone present as a contaminant was low and insufficient to stimulate lactation. At the higher levels injected, many of the rabbits were unable to tolerate the thyrotropic hormone except during periods of cold weather when the excess heat produced could be dissipated.

It might be said, however, that the lactogenesis of the thyrotropic fraction was being masked by the excess of the thyrotropic hormone. The excess metabolism induced would be unfavorable to the lactation process. One might say that if the thyrotropic hormone were removed, the other factors would be able to show their influence. As we did not have a preparation free of the thyrotropic hormone, yet containing the other factors, it was decided to thyroidectomize the rabbits and thus free them of the metabolic upset which follows large injections of this fraction.

Lactogenesis in Thyroidectomized Rabbits. Five rabbits were thyroidectomized, three days before the end of pseudo-pregnancy. The injection of 5 mg. of the "thyrotropic and other hormones" extract per 100 gms.

TABLE 2

Lactation response in thyroidectomized pseudo-pregnant rabbits with the "thyrotropic and other hormones" fraction

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman-Turner thyrotropic units (Guinea pigs)	McShan-Turner lactogenic units	Gardner-Turner rating of lactation
	gms.	*mg.*	*mg.*			
1	2825	5	140	560	14	++
2	3020	5	150	600	15	0
3	3710	5	185	740	18.5	0
4	4100	5	205	820	20.5	0 to +
5	3360	5	170	680	17	++
1	2680	10	270	1080	27	+
2	2800	10	280	1120	28	++
3	3600	10	360	1440	36	++
4	3170	10	320	1280	32	++
5	3500	10	350	1400	35	+
6	3340	10	330	1320	33	+
7	3030	10	300	1200	33	+

body weight initiated (++) lactation in two animals but little or none in the other three (table 2).

Seven other similar rabbits were injected at the rate of 10 mg. per 100 gms. body weight. These animals all showed at least duct lactation (+) with three showing further progress (++) in the filling of the gland with milk. As the units of the "lactogenic" hormone present as a contaminant in the extract were still far less than that required to induce a similar stage of lactation in normal animals, it would appear that other factors in the pituitary were able to supplement and intensify the apparent activity of the lactogenic hormone present.

A tentative theory to explain this phenomenon follows: On the assumption that a true lactogenic hormone is required to condition the epithelial cells of the mammary gland to synthesize milk, it indicates that without the hormone no lactation would result. On the other hand, it might require less lactogenic hormone to develop a certain stage of filling of the gland, if, once the cells were activated, other hormonal factors stimulated the rate of milk secretion by the epithelial cells.

As the "thyrotropic and other hormones" fraction contained considerable amounts of the factor which elevates the blood sugar, it seems reasonable to believe that this hormone would augment lactation once established and at the end of the test period of six days show a degree of lactation greater than would be obtained by an equivalent amount of pure lactogenic hormone. As a matter of fact it seems possible that the pituitary contains several lactation augmenting hormones. Even the thyrotropic hormone in suitable dosage would be expected to augment lactation considering the demonstrated influence of thyroxine on lactation (12, 13).

Supplementing Effect of Pituitary Hormones on Lactation. If one assumes that the initiation of lactation by the epithelial cells of the mammary gland is stimulated by the lactogenic hormone whereas other pituitary hormones augment the rate of the lactation process thus established, it should be possible to obtain higher lactation ratings in the pseudo-pregnant rabbits with a minimum of lactogenic hormone if supplemented with the other fraction. It was decided, therefore, to determine the effect of injecting one mg. of the lactogenic hormone, which when injected alone produced an average lactation rating of 1.67, with one mg. of the "thyrotropic and other hormones." The seven rabbits which survived showed a slight augmentation of lactation, averaging 1.86 (on the Gardner-Turner rating).

In a second group of five rabbits, the "thyrotropic" fraction was increased to two mg. per 100 gms. body weight, the lactogenic remaining the same as previously (1 mg.). These animals showed (++) to (+++) ratings, averaging 2.60. In other words, with lactogenic hormone alone about 260 McShan-Turner pigeon units were required to induce (+++) lactation, whereas in these experiments about 100 units less of the lactogenic hormone

were required to produce the same degree of gland filling when the other hormones of the pituitary supplemented its action.

DISCUSSION

Azimov and Krouze (14) were the first to point out the apparently paradoxical fact that crude bovine pituitary preparations were able to increase established lactation in dairy cattle to a greater extent than the more highly purified lactogenic hormone fraction. Folley and Young (1) confirmed this observation and further obtained a substantial temporary increase in milk volume with cows injected with a "thyrotropic" preparation which contained no detectable amount of the substance that stimulates growth of the pigeon crop gland. From these and other observations Folley (15) has questioned the specificity of the lactogenic hormone when assayed by the pigeon crop gland. It is his opinion that extracts which stimulate the secretion of normal milk in mammals should be described as containing the lactogenic substance.

The writers (3) have recently presented evidence which they interpret as indicating that the extract of the pituitary which is commonly called the "lactogenic hormone" and which possesses the ability to proliferate the pigeon crop gland also possesses the ability *to initiate* lactation in pseudopregnant rabbits to a high degree. In the present paper it was shown that the extract remaining, rich in the thyrotropic and other hormones but containing little "lactogenic" hormone would only rarely *initiate* lactation in dosages as high as could be tolerated.

It is the opinion of the writers that the confusion which the papers of Folley and Young may have created in the minds of some may be eliminated by a suitable definition of the term *lactogenic*. We suggest that the term *lactogenic* be reserved for the hormone or hormones which have the ability to initiate secretory activity in the epithelial cells of the mammary gland as contrasted with the terms *galactogogue* or *galactopoetic* for substances which have the ability to augment the rate of established lactation. By these definitions, the hormones of the anterior pituitary and of other endocrine glands which directly and indirectly influence the lactation process may be divided into two groups. The lactogenic hormone is believed to act directly upon the epithelial cells of the mammary gland initiating and maintaining the secretory activity of the cells in the presence of suitable amounts of the milk precursors. This hormone will initiate lactation in the glands of rabbits, guinea pigs and other animals when they are conditioned by the mammogenic hormones. It also stimulates the proliferation of the pigeon crop gland. The experiments with hypophysectomized animals (16) indicate the need of the hormone continuously during lactation. It is true that the lactogenic hormone may also augment established lactation when the rate of secretion of the hormone is less than optimal in relation to the precursors

TABLE 3

Lactation response in normal pseudo-pregnant rabbits with supplementing effect of pituitary hormones

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman-Turner thyrotropic units (guinea pigs)	McShan-Turner lactogenic units	Gardner-Turner rating of lactation
	gms.	*mg.*	*mg.*			
1	3260	1 Th*	33	132	3.3	
		1 Lac†	33		165.0	++
2	3430	1 Th	34	136	3.4	
		1 Lac	34		170.0	+
3	3220	1 Th	32	128	3.2	
		1 Lac	32		160.0	+
4	2820	1 Th	29	116	2.9	
		1 Lac	29		145.0	+++
5	3460	1 Th				
		1 Lac				Died 2nd day
6	2700	1 Th	27	108	2.7	
		1 Lac	27		135.0	+++
7	4230	1 Tt	42	168	4.2	
		1 Lac	42		210.0	+
8	2780	1 Th	28	112	2.8	
		1 Lac	28		140.0	++
Ave. of seven	3206	1 Th	32.1	128.4	3.2	1.86
		1 Lac	32.1		160.5	
1	3910	2 Th				
		1 Lac				Died 1st day
2	2970	2 Th	60	240	6.0	
		1 Lac	30		150.0	+++
3	3520	2 Th	70	280	7.0	
		1 Lac	35		175.0	+++
4	3190	2 Th	64	256	6.4	
		1 Lac	32		160.0	+++
5	4270	2 Th	86	334	8.6	
		1 Lac	43		215.0	++
6	2690	2 Th	54	216	5.4	
		1 Lac	27		135.0	++
7	3910	2 Th				Died 5th day
		1 Lac				++ rating
Ave. of five	3328	2 Th	66.8	267.2	6.7	2.60
		1 Lac	33.4		167.0	

* Th = "thyrotropic and other hormone" fraction.
† Lac = "lactogenic hormone" fraction.

of milk. Consequently, in established lactation it is impossible to distinguish the lactogenic from galactogogic hormones.

The mode of action of the galactogogic hormones is believed to be indirect through their ability to mobilize larger quantities of the precursors of milk and to increase the metabolism of the cells. These hormones affect many bodily processes and their ability to augment the rate of milk secretion is solely due to the ability of the mammary gland to take advantage of

the improved general metabolism of the body. In other words, in the absence of the lactogenic hormone no lactation would occur even with increased mobilization of milk precursors.

The writers have reason to believe that the anterior pituitary secretes a number of hormones which individually and collectively have the ability to augment the lactation rate. Further, it has been suggested that the differences in the productive ability of dairy cattle is due in part to the differences in the rate of secretion of these hormones and the glands and hormones which they influence (17). The outstanding example of this relationship is the thyrotropic hormone which stimulates the secretion of thyroxine. Thyroxine in turn has been shown to have a marked influence in augmenting the rate of established lactation (13). The pituitary hormones also influence the adrenal gland and the metabolism of fat, carbohydrate, protein and mineral matter. As these factors are separated from the mixture of hormones, the augmentation of lactation due to their joint action may be separately evaluated.

SUMMARY

A study is reported with the "lactogenic hormone" and the "thyrotropic and other hormone" fraction of the anterior pituitary on lactogenesis in the pseudo-pregnant rabbit.

When the "lactogenic hormone" fraction was injected at the rate of 1 mg. per 100 grams body weight (+) to (++) glands were obtained, the average rating being 1.67. When 1.5 mg. was given (+++) to (++++) glands were obtained the average rating being 3.17, which is considered in the range for a unit response. At this level approximately 52 mg. containing 260 McShan-Turner pigeon units were required.

A group of rabbits injected with 2 mg. per 100 gm. body weight of the "thyrotropic and other hormone" fraction showed no evidence of lactation. At the 5 mg. and 10 mg. levels six animals in each group survived the test period. Only one rabbit in each group showed any evidence of lactation.

In a group of five thyroidectomized rabbits injected with 5 mg. of the "thyrotropic and other hormone" fraction per 100 grams body weight (++) lactation was initiated in two animals but little or none in the other three. Another group of seven thyroidectomized rabbits injected at the 10 mg. level gave mostly (+) to (++) glands, the average rating being 1.43. All the animals survived the test period.

The average gland rating was increased from 1.67 for 1 mg. of lactogenic hormone per 100 grams body weight, to 1.86 when supplemented by 1 mg. of the "thyrotropic and other hormone" fraction. When the level of injection of the latter was 2 mg. the average gland rating was increased to 2.60.

These results are taken to indicate that the primary function of the lac-

togenic hormone, which also possesses the ability to proliferate the pigeon crop gland, is to initiate and maintain established lactation. Extracts rich in the thyrotropic and other hormones, but containing only traces of the lactogenic hormone, do not possess the ability to initiate lactation in doses as high as could be tolerated. This fraction, however, has a supplementing effect on established lactation.

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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

586. Relationship of Curing Temperatures to Quality of American Cheddar Cheese. H. L. WILSON, S. A. HALL, AND WM. T. JOHNSON, JR., Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture.

A report of a study to determine the effect of curing temperatures on the quality of American Cheddar cheese in which one hundred and sixteen vats of milk of good quality were made into cheese, and the four duplicate cheeses made from each vat were stored as follows: One was held for 6 months at 34° F.; one for 3 months at 50° plus 3 months at 34°; one for 6 months at 50°; and one for 3 months at 60° plus 3 months at 34°.

The following results were obtained:

In the group stored for 3 months at 50° plus 3 months at 34° F., and also in the group stored 6 months at 50°, the majority of the cheeses were better in quality than the duplicates (or controls) that were stored 6 months at 34°. But in the group stored 3 months at 60° plus 3 months at 34°, the majority of the cheeses were poorer in quality than the duplicates stored 6 months at 34°.

Of the 116 cheeses that were stored 6 months at 50° F., about 80 per cent had a score of 92 or more at the end of the storage period and the other 20 per cent had a score of less than 92. Of the high-scoring group, 81 per cent were better in quality than, or as good as, the duplicate that was stored 6 months at 34°; whereas, of the lower-scoring group, only 26 per cent were better than, or as good as, the duplicate stored 6 months at 34°.

From these data, it appears that if a cheese is destined as a result of some inherent defect in the making process to have a score below 92, it will have a relatively higher score if it is held at 34° F. than if it is cured at 50°.

When cheese is held 6 months at 34° F., the percentage that will score 92 or better will be about the same whether the moisture content is above or below 38 per cent. When cheese is cured at any of the three higher temperatures used in this experiment, however, the percentage scoring 92 or better will be from 5 to 15 per cent in favor of the low-moisture cheese.

Cheese made from milk of good quality and by methods which insure cheese of good quality can be cured at temperatures as high as 50° F. with reasonable certainty of developing a clean and characteristic Cheddar flavor.

From past observations, cheese made from bacteriologically poor milk or so manipulated that these is a tendency to develop acid, bitter, or other off-flavors, should be stored at 34° F. in order to retard the development of these defects as much as possible.

587. A Technique for Perfusing Excised Bovine Mammary Glands. W. E. PETERSEN, J. C. SHAW, AND M. B. VISSCHER, University of Minnesota.

Details are given for a suitable apparatus that will produce the necessary pulsations and the desired blood pressure, and has provisions for aeration of the venous blood. Care in excising and handling the mammary gland as well as the general features involved in conducting a perfusion experiment are discussed. W.E.P.

588. Some Factors Involved in Efficient Milking. KENNETH MILLER AND W. E. PETERSEN, University of Minnesota.

Data are presented to show the effect upon milk and fat production of lengthening the interval between milking and stripping; stimulating the cow to let down the milk sometime before the milking begins and increasing the length of time involved in the milking process. Delaying the time when cows are stripped following the milking machine has little or no effect upon milk and butterfat production. Stimulating the cow to "let down" milk 20 minutes before milking decreased both milk production and fat percentage and had a tendency to "dry off" cows. Lengthening the time required for milking had the same effect as stimulating the "let down" before the milking begins. The observed effects are tentatively explained as being due to a dissipation of the oxytocic principle before the milk is emptied out of the alveoli and ductules of the gland. W.E.P.

589. The Relationship of Ascorbic Acid to Reproduction in the Cow. PAUL H. PHILLIPS, H. A. LARDY, P. D. BOYER, AND GEORGE M. WERNER, Departments of Biochemistry and Dairy Husbandry, University of Wisconsin, Madison.

The results of these experiments have shown (1) that the ascorbic acid content of plasma averaged 0.39 mg. per cent for cows of all breeds studied, (2) that there is a difference between breeds with the Holstein lower than the Guernsey, (3) that there is a higher concentration of ascorbic acid in the plasma of the cow in mid to late estrum than there is in anestrum, (4) that generally speaking there is no difference between good and poor breeders in their peak concentrations of ascorbic acid during estrum, (5) that the subcutaneous ascorbic acid therapy of "hard to settle" cows results in a positive response in 60 per cent of the cases treated, (6) that ascorbic acid therapy does not correct cases of cystic ovary or other anatomical abnormalities, and (7) that α-tocopherol proved ineffective in restoring tone to a toneless uterus.

From these researches the conclusion is evident that ascorbic acid is intimately associated with the early phases of the reproductive processes and

it can be successfully used as a therapeutic measure in treating certain types of sterility in the cow.

BOOK REVIEWS

590. Judging Dairy Cattle. E. S. HARRISON, Cornell University. March, 1940. 132 pages. Price $2.75. John Wiley and Sons, Inc., New York.

This book makes a valuable addition to the literature on dairy cattle breeding and judging. It is beautifully illustrated. The photographs used are those produced by H. A. Strohmeyer, Jr., and J. T. Carpenter, Jr. The subjects were well selected and the photography is excellent. The author relies on the pictures to explain his points and to demonstrate the characteristics under discussion. The subject matter is brief and to the point. This book should be of interest to teachers, dairy cattle judges and breeders.

The author introduces the main theme of the book with a brief statement of his philosophy about dairy cattle breeding and the importance of type in a constructive breeding program. He makes it clear that he believes that first and foremost a dairy cow must produce large amounts of milk and fat year after year. He questions the value of short time records or even single lactations as a measure of a cow's ability. He feels that a great majority of the high producing cows which continue to produce year after year are cows of acceptable type. He draws these conclusions from his immediate experience with the Cornell University dairy herd and from a study of the production records of show cattle and the production records of cattle officially classified.

In chapter one the author reproduces the score cards and the true type pictures of the cows of the five dairy breeds. He discusses the value of the score cards and use that may be made of them.

He illustrates with photographs, in chapter two, the difference between desirable and undesirable heads and necks. The pictures are accompanied with a minimum of explanatory material.

Chapter three deals with the mammary system. Pictures are extensively used to show in great detail the udder, flank and milk veins, both side and rear views. They show the common udder faults, accompanied by brief explanations. In this chapter, two excellent udders are shown dry and in full milk flow. Here the author discusses the characteristics to be looked for in the dry and lactating udder.

In chapter four, the legs and feet come in for consideration. The author stresses the desirability of strong legs properly placed and set. The common faults, such as crooked hind legs, close hocks, overly straight hind legs and weak pasterns, are all well portrayed and described. Several pages are devoted to the need of giving feet the proper care. The author shows what happens when feet are neglected and allowed to get long and out of shape.

Several pictures are used to show just how a cow's foot should be trimmed. This is an especially good demonstration and shows the methods which all dairymen might well use.

Chapter five deals with the chest, chine, crop and shoulders. The author gives reasons for the desired characters and demonstrates effectively with photographs the correct and incorrect form. The photographs show the chest conformation and its relation to the set of the front legs.

Chapter six is devoted to reasons and placings. Here, pictures show front, rear and side views of several pairs of cows and the author gives his reason for the placing of each pair.

Chapter seven is given over to the problem of heifer judging. The author here spends considerable space explaining the characteristics to look for in the undeveloped udder. He emphasizes the need to consider the size, shape and placement of teats as well as udder attachment fore and rear. This chapter includes an interesting group of photographs of two noted show cows portraying their development from calfhood to maturity.

The last chapter deals with judging of bulls. The author recognizes the fact that the real value of a bull is determined by his get. In the show ring he feels that the bull of sharp angular type should be given preference since that corresponds to the type desired in cows. Here as elsewhere, the author uses numerous pictures to illustrate his meaning. C. L. Blackman

591. **Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists; 5th Edition.** Published by Association of Official Agr. Chemists, Washington, D. C. 757 pages, $5.00.

This handbook of analytical procedures published about every five years is a compilation of tentative and official methods for the analysis of food and agricultural products. Analytical methods are proposed by a referee of the Association for acceptance as tentative methods after having been subjected to collaborative study in various laboratories. Tentative methods, after having been given further study and consideration may become, upon approval by the Association, official methods. The official methods are acceptable for court action; tentative methods are acceptable for court action in the absence of official methods for the same analysis.

The book consists of 40 chapters plus (25) tables, appendix and index. Chapters of particular interest to the dairy industry are those on beverages; malt beverages, sirups and extracts, and brewery materials; cocoa bean and its products; coloring matter in foods; dairy products; eggs and egg products; flavoring extracts; fruits and fruit products; metals in foods; oils, fats and waxes; sugars and sugar products; vitamins; waters; brine and salt.

New additions to the sections on Dairy products are the phosphatase

test (Gilcreas), the bioassay technique for the vitamin D line test, estimation of citric acid in malted milk powder, determination of mold mycelia in butter, and estimation of solids in milk by specific gravity measurements. Revision and extension was made of methods for detection of gums in cheese and the preparation of butter samples for estimation of moisture content. Tentative methods which have become official are: the estimation of fat in malted milk, and ash and total chlorides in cheese. Approximately 100 tests (primarily proximate analyses) for dairy products alone are detailed.

Tentative methods for estimating zinc colorimetrically, for determining the thiocyanogen number of fats and oils, and a micromethod for reducing sugars are included in other sections. Microbiological methods for examination of frozen egg products, and sugar are also included.

The new section on vitamins includes only assay methods for vitamin D in milk and in concentrates for poultry feeds.

Despite enlarged scope and increased material, the convenient size of this famous "Book of Methods" has been retained by economy of verbiage, without affecting its usefulness. K.G.W.

BACTERIOLOGY

592. The Rosenthal Anaerobic Method. A. A. Miles. Lancet, *239:* 7. 1940.

When "technical" chromium powder was substituted for pure chromium powder in the Rosenthal method a gas was evolved which was bactericidal to some species of spore-bearing anaerobes and bacteriostatic to others. The toxic gas was believed to be hydrogen sulphide. J.F.C.

BUTTER

593. New Aids to Better Cream. C. H. Parsons, Swift and Co., Chicago, Ill. Nat. Butter and Cheese J., *31:* 8, 14. 1940.

It was observed that mixed lots of cream when held under adverse conditions tend to be nearer the quality of the poorest component rather than an average of all lots. A series of experiments which were conducted to simulate farm conditions show that the quality of cream is somewhat better maintained when containers of minimum surface area are used and when the cream is not stirred during the gathering period, rather than when held in the conventional cream can and stirred after each addition of cream. Investigations should be initiated to simplify and improve methods of maintaining quality of cream on the farm and should deal with such problems as cleaning and sterilization of separator and utensils, exclusion of extraneous matter, cooling methods, and design of cream containers. W.V.P.

594. What is the Cost of a Pound of Butter? G. M. PELTON, Swift and Co., Chicago, Ill. Nat. Butter and Cheese J., *31:* 8, 10. 1940.

Every creamery operator should determine costs in his own plant. Kinds of costs and expenses are: procurement expenses, plant delivered cost and processing expense. These costs and expenses are broken down to show specific items which must be included in calculating cost per unit of production. Operators should figure test costs frequently to guard against unexpected changes and are shown how to make the calculations. No actual costs are given. W.V.P.

595. Incubation Test as an Indication of the Keeping Quality of Butter. H. B. NAYLOR AND E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Nat. Butter and Cheese J., *31:* 9, 10. 1940.

Ten churnings which were laboratory made under conditions to insure the best possible keeping quality and 28 churnings from 4 creameries were subjected to the incubation test for keeping quality. Salted samples from each churning were tested for moisture and salt. Salted and unsalted samples were analyzed for total and caseolytic bacteria, pH and scored for flavor when fresh, after 7 and 14 days storage at 60°, after 3 months at 0° F. and after 10 days at 60° F. following the 0° F. storage period. Salted and unsalted butter made in the laboratory showed little or no flavor deterioration under any conditions. The incubation test predicted fairly well the keeping quality of the high grade butter. The pH of salted samples (9.5 per cent NaCl in the brine) was lower than the unsalted, probably due to the NaCl effect on the hydrogen ion. There was no correlation between either pH or bacterial content and keeping quality of high grade butter. The incubation test was quite reliable for predicting keeping quality of commercial butter samples. The salted samples kept best. Flavor and pH values on unsalted butter dropped during incubation both before and after cold storage but pH in salted samples did not change regardless of flavor changes. Only unsalted samples seemed to show deterioration in flavor due probably to caseolytic types of Gram-negative, rod-shaped organisms.

W.V.P.

596. Preparation and Care of Starters. Article 1. MICHAEL B. MICHAELIAN, Verley Products Corp., Chicago. Nat. Butter and Cheese J., *31:* 9, 16. 1940.

This article defines bacteria, yeasts and molds and explains some terms commonly used in discussing starter cultures. The cause of aroma and flavor in starters is reviewed. The creatine test for acetylmethylcarbinol plus diacetyl and its application in judging starters are described.

W.V.P.

597. Factors Affecting Mold Mycelia Content of Butter. W. L. SLATTER, Ohio State Univ., Columbus, Ohio. Nat. Butter and Cheese J., *31:* 9, 50. 1940.

Representative samples of first and second grade creams were obtained from individual producers in central Ohio. Acidity, fat, mold, yeast and mold mycelia content were determined on each raw cream sample. Samples were neutralized, pasteurized, cooled and churned. Mold, yeast and mold mycelia content of the butter were determined. Mold mycelia contents of butter and the cream from which it was made are not closely related. From questionnaires filled out by each producer it was determined that butter with the lowest mold mycelia content is produced by large herds where cream is cooled, kept cold and delivered at least twice a week. W.V.P.

CHEESE

598. Use of By-products in Making Cheese Spreads. C. R. BARKER, Oak Park, Ill. Nat. Butter and Cheese J., *31:* 8, 64. 1940.

Cheese processed with condensed or dried whey or condensed or dried skimmilk forms an excellent cheese food. Five to 10 lbs. of whey solids can be used per 100 lbs. of finished product; more whey solids tend to produce lactose crystals. Desirable spreading properties are produced with 42 to 44 per cent water. When skimmilk solids are used the casein builds up cheese body so that cream, butter or water must be added to give spreading properties. The pH of cheese mixtures should be adjusted to 5.5 to 5.9 with citric or phosphoric acids to avoid putrefactive spoilage. W.V.P.

599. Retarding Mold in Cheese. ANONYMOUS. Nat. Butter and Cheese J., *31:* 8, 12. 1940.

Frederick W. Miller, Jr., E. I. du Pont de Nemours and Co., conducted tests to show the effects of propionic acid, and sodium and calcium propionates on growth of mold on cheese cuts enclosed in cellophane. Half pound wedge-shaped pieces of cheese were first exposed to air contamination then immersed in the treating solution, drained, wrapped and sealed in 300 MST Cellophane bags, and finally stored at 58° to 60° F. and 90 per cent relative humidity. The results show that propionic acid is about twice as effective as the solution of salts. Mold growth is retarded by more concentrated solutions, longer immersion periods, shorter draining intervals before wrapping, tight fitting wrappers and limited amounts of contamination. Treatment in 8 per cent propionic acid for 15 seconds and a 4-minute draining period produces 18 to 21 days mold delay. Excessive concentrations or prolonged treatments with acid or salt solutions cause undesirable changes in color and surface flavor. W.V.P.

600. Process Cheese on a Small Scale. C. R. BARKER, Oak Park, Ill. Nat. Butter and Cheese J., *31:* 9, 15. 1940.

Prospective manufacturers should have a knowledge of bacteriology, chemistry, dairy husbandry, cheese manufacture and selling. Recommended pieces of equipment for a single story operation are a steam jacketed kettle, a large powerful grinder, an inexpensive filling machine to match the kettle in size, and a conveyor for boxes. W.V.P.

601. Fifty Years of Cheese Making. C. F. DOANE, Salem, Oregon. Nat. Butter and Cheese J., *31:* 1–9. 1940.

Information from fifty years of close contact with the cheese industry is reviewed in a series of 9 articles published in 9 issues. I (*31:* 1, 30. 1940) The granular type of cheese made 50 years ago has practically disappeared along with the curd sink and blade curd knife. The hot-iron test made development of the cheese industry possible and the whey separator made factories more efficient. II (*31:* 2, 30. 1940) High ideals of quality have been lowered by the sale of green cheese, by excessive yields and lack of payment for a superior cheese. The consumer does not recognize inferior quality in green cheese. III (*31:* 3, 56. 1940) Cheese making has been favored by climatic conditions in northern states. Pasteurization of milk has been introduced as a necessity in southern states and New Zealand but the value of the process can be questioned because of ripening difficulties. Process cheese lacks positive quality but sells because it is uniform, advertised, packaged and mild in flavor. IV (*31:* 4, 42. 1940) Good milk is necessary for good cheese. Helpful tests to measure quality are the curd test, gas tubes and methylene blue test. Tillamook has made better returns because of high quality milk and this can be done anywhere in the United States not affected by long hot spells. Utensils, strainers and milking machines may contaminate milk but every factory patron must produce good milk and can do so easily at practically no extra expense. V (*31:* 5, 14. 1940) Acid development is commonly linked with desirable quality and control of fermentations in cheesemaking. But acid development should be restricted for best results. Even in control of gas, acid may really be less effective than commonly supposed. Low acid development in making lengthens the time of curing but improves cheese quality, particularly the flavor, and decreases chances of loss from excess acid. VI (*31:* 6, 36. 1940) Standards of composition compatible with good quality are fixed by law. It is the privilege and duty of skillful makers to control cheese composition. Such control can be attained through proper manipulations of acid and heat plus daily tests for moisture. Where correct standardization of fat is practiced there is no loss of quality. VII (*31:* 7, 12. 1940) Canning of natural cheese for curing retains volatile flavors both good and bad and the flavors of the cheese are thus intensified even

when a venting valve is used to release partially the pressure of gas formed during normal curing. A flavor called salty-acid or metallic in canned cheese is a permanent defect but putrid flavor and odor tend to disappear on exposure to air. Even good milk may produce cheese with some off flavors when it is ripened in cans. Pasteurized milk has been used to commercialize cheese canning but this treatment does not always produce the desirable flavors of raw-milk cheese. The salt-acid criticism may be due to the natural salts of milk rather than to bacterial changes. The addition of water to the milk removes some of the milk salts and the resulting cheese is improved in salt-acid flavor and produces gas for a shorter time in curing. The characteristic putrid flavor is eliminated when the canned cheese is ripened under a slight vacuum. The use of a square hoop and can to form and cure many small rectangular packages of cheese at moderate cost may be a desirable development but the plan of merchandising is not yet clear. VIII (*31:* 8, 30. 1940) The Tillamook cheese industry is an unusual cooperative effort which has prospered because of a good manager, favorable climate, control of milk and cheese quality, careful grading and uniformity of product. IX (*31:* 9, 12. 1940) Starter organisms help develop acid more rapidly in cheesemaking but may not control undesirable types of bacteria. Starter added to excess spoils Cheddar cheese eventually if not immediately; 0.5 per cent is more satisfactory than 0.75 per cent which is about the safe limit. Cheese makers should select starters carefully because of variations in the results attained with different cultures.

W.V.P.

CHEMISTRY

602. A Biological Assay of Riboflavin in the Liver of the Cow, Calf, Sheep, Lamb, and Hog. O. B. Saffry, H. S. Cox, B. L. Kunerth, and M. M. Kramer, Kansas State College, Manhattan, Kansas. J. Nutrition, *20:* 169–174. 1940.

The riboflavin content of beef, calf, lamb and pork livers was determined according to the Bourquin-Sherman method. The riboflavin values per 100 grams were as follows: Lamb-liver, 4950–5400 mg.; mutton liver 4350 mg.; calf liver, 3450–4350 mg.; beef liver, 2850–3450 mg. and pork liver 2700 mg. Liver samples purchased in the winter season were slightly higher in riboflavin than samples purchased in the fall. C.F.H.

603. The Effects of High Pressure on the Activity of Pepsin and Rennin. Jason E. Matthews, Jr., R. B. Dow, and Arthur K. Anderson, Dept. of Agr. and Biological Chem., and Dept. of Physics, Pennsylvania State College, State College, Pa. J. Biol. Chem., *135:* 697. 1940.

The activity of pepsin and rennin decreased with pressure increase at

constant exposure time and was completely destroyed at pressures ranging between 5000 and 6000 kilos per sq. cm. Increase of exposure time at constant pressure likewise decreased the activity but to a lesser degree. Inactivation of pepsin and rennin by pressure depended strongly on the temperature. Loss of activity appeared to be dependent on certain buffers and the pH.

Both enzymes were heavily coagulated by pressure treatment at 10,000 kilos per sq. cm., a result that appeared to be the same as when the solutions were heated to 100° C. Although the product of denaturation by pressure appeared similar to that which had been denatured by heat the energy relationship is different in the two processes. V.C.S.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

604. It Pays to Condense. P. S. LUCAS, Michigan State College, E. Lansing, Mich. Nat. Butter and Cheese J., *31:* 9, 36. 1940.

Practical, efficient equipment for concentrating small amounts of milk is now available at reasonable cost. Small milk plants should consider the use of such equipment to dispose of milk solids profitably. Omitting overhead, the cost of condensing 30 cans of skimmilk to 10 cans in a 3-foot pan were estimated to be $0.79 for coal, $0.12 for electricity, $0.60 for water and $1.13 for labor. W.V.P.

DISEASE

605. The Control of Streptococcic Mastitis in a Certified Herd. O. W. SCHALM, Univ. of Calif., Berkeley, Calif. Cert. Milk, *15:* 167, 11. March, 1940.

This paper presents the methods employed in eradicating streptococci mastitis from a certified herd. The program was based on the assumption that every cow shedding *Streptococcus agalactiae*, regardless of the condition of its udder, is a potential source of infection of the clean cows in the herd. Once a cow was classified as infected, it remained in that classification irrespective of subsequent findings. W.S.M.

606. Tubercle Bacilli of the Bovine Type in Skin Lesions. Editorial, Am. J. Pub. Health, *30:* 551–552. 1940.

This editorial discusses an article by an investigator in Western Poland who finds that the bovine type is more frequent in skin tuberculosis than in any other organ of the body. *Lupus vulgaria* is the most frequent form of skin tuberculosis in Poland and it is estimated that there are some 25,000 cases of lupus in that country. The cattle in Poland have a rather high per cent of tuberculosis and in Warsaw 50 per cent of the market milk is contaminated with the tubercle bacillus. M.W.Y.

607. The Deterioration of the Bovine Udder in the Absence of Streptococci. E. G. HASTINGS AND E. H. PETERSON. J. Agr. Res., *60:* 3. Feb., 1940.

Observations were made to determine the chemical composition and bacterial flora of the foremilk of 11 Holstein-Friesian animals for the first three lactation periods. The animals were isolated from other animals and fed rations typical for many dairy farms. The chlorine and catalase values were found to increase during a lactation period and also increased from one lactation period to the next. By the middle of the third lactation period the average percentage of chlorine and catalase was above the values usually considered normal. The results of the bacteriological studies supplied no explanation for the chemical changes in the milk. The udders of each animal remained free of streptococci and no other organisms were found consistently or to any great extent during the three lactation periods. The authors suggest that the causes of mastitis or of deterioraion of the udder may be more complex than now supposed and indicate the need for more detailed and prolonged study of the subject. W.J.C.

FOOD VALUE OF DAIRY PRODUCTS

608. Nutritional Aspects of Milk. W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Cert. Milk, *15:* 167, 3. March, 1940.

A discussion is given on the use of our newer knowledge of nutrition for making milk a better food than it already is. W.S.M.

609. Iron Utilization in Dogs on Milk Diets. D. V. FROST, C. A. ELVEHJEM, AND E. B. HART, Dept. of Biochem., Univ. of Wisconsin, Madison, Wis. J. Nutrition, *19:* 311–320. 1940.

The ratio of requirement of iron to copper is only about 5 to 1. Iron is absorbed and stored, but does not become available for hemoglobin building unless adequate copper is supplied.

Some degree of caution should be urged regarding the use of cobalt as a hematopoietic agent. Although in many instances small amounts of cobalt have appeared to stimulate hematopoiesis in dogs on milk diets, a clear-cut need for this element has not yet been established. C.F.H.

610. Ascorbic Acid Content of Goat's Milk and Blood: Influence of Ascorbic Acid and Injections and Diet. M. S. RICHMOND, G. H. SATTERFIELD, C. D. GRINNELLS, AND W. J. DANN, Univ. of N. Carolina, Raleigh, and Duke Univ., Durham, N. Carolina. J. Nutrition, *20:* 99–108. 1940.

Goats were fed a ration consisting of alfalfa or lespedeza hay and a grain mixture consisting of 4 parts of corn, 3 parts cottonseed meal, 2 parts wheat

bran and 1 part oats. The animals were turned to pasture in season. Blood and milk samples were taken at approximately weekly intervals.

The ascorbic acid in blood ranged between 0.6 and 0.8 mg. per 100 ml., and in milk between 0.5 and 2.0 mg. per 100 ml. There was no consistent relationship between the amount of ascorbic acid in the milk and that in the blood. The alternate feeding of a normal and an ascorbic acid-free diet to four goats indicated that the ascorbic acid of the blood is not closely dependent on the amount of ascorbic acid in the diet.

The injection of 1 or 2 gm. of ascorbic acid intraperitoneally resulted in a large increase in blood and urine ascorbic acid, and a slight increase in milk ascorbic acid. C.F.H.

611. **The Utilization of Calcium in Carrots, Lettuce and String Beans in Company with the Calcium in Milk.** J. B. SHIELDS, B. W. FAIRBANKS, G. H. BERRYMAN, AND H. H. MITCHELL, Div. of Animal Nutrition, Univ. of Illinois, Urbana. J. Nutrition, *20:* 263–278. 1940.

Rats were used for experimental animals in a study of the relative availability of calcium from various sources. The results indicated that the commercial desiccation of milk does not appreciably impair the value of its calcium in the nutrition of growing rats. The calcium of milk was definitely better utilized than the calcium of fresh carrots, fresh lettuce and fresh green string beans, which was 85, 80, and 74 per cent respectively as available as the calcium of milk.

The steam cooking of carrots and the commercial canning of green string beans do not modify appreciably the value of these vegetables as sources of dietary calcium. C.F.H.

HERD MANAGEMENT

612. **Raising Calves on Wire Floors.** H. H. TUCKER, Educational and Research Bureau for By-Product Ammonia, Columbus, Ohio. Cert. Milk, *15:* 168, 9. April, 1940.

This article describes in detail the construction of wire floors for the calf pen. The virtues for this new type of floor are, (1) the labor required to care for calves is greatly reduced over that normally required; (2) a great saving in bedding; and (3) an increased growth and development of the calves, and decreased losses due to death and sickness. W.S.M.

MILK

613. **Receipts, Utilization, and Prices of Milk and Cream in Maine Milk Control Areas.** GEORGE F. DOW, Maine Agr. Exp. Sta. Bull., 399. March, 1940.

A presentation of developments in the public control of milk prices up

to July, 1939, an analysis of milk distributors' records submitted to the Maine Milk Control Board up to December 31, 1937, and a study of other readily available information pertaining to milk distribution in Maine. Twenty thousand, four hundred and twelve monthly reports of 927 distributors were included in the study of 29 areas, comprising receipts and disposal of 109, 392,867 quarts of milk and 4,936,549 quarts of cream. A study of milk control operation in the state of Maine included in this bulletin is of interest because it is quite largely based upon the business of the producer-dealer and small dealer type rather than that of the large milk dealer. Average surplus in markets included in the study was 16.0 per cent for milk and 17.3 per cent for cream. A flat price plan was used for paying producers in the smaller markets where producer-distributors handled most of the milk. A classified price plan was used in eleven of the larger markets where large quantities of milk were purchased. The average price paid producers per quart of 4.0 per cent milk under a classified price plan was 5.9 cents for fluid milk, 3.9 cents for surplus milk, and 5.5 cents for all milk. The average price paid producers for heavy cream in most markets was $1.75 per gallon. None of the markets had regulations providing for base ratings or equalizations of sales because the amount of surplus milk was relatively small. The average spread for distributing cream was three to six times higher than for milk, indicating relatively wide spreads and high profits per unit of cream sold. L.M.D.

614. Why Milk?—From the Standpoint of Human Health and Economics. E. V. McCollum, Johns Hopkins University, Baltimore, Md. Cert. Milk, *15:* 169, 3. May, 1940.

The importance of milk in the diet from the standpoint of proteins, minerals and vitamins is discussed. It is pointed out that nutritionists are gradually impressing upon the public that prevention and not cure of deficiency diseases is the object to be sought. W.S.M.

615. Conditions Influencing Cream Volume of Raw and Pasteurized Milk. C. L. Roadhouse and J. L. Henderson, Univ. of Calif., Davis, Calif. Cert. Milk, *15:* 168, 7. April, 1940.

It was found that the rapidity and extent of cooling have the greatest influence on the cream layer volume of both raw and pasteurized milk. However, the temperature to which the milk is cooled to give the maximum cream layer is not the same for both kinds of milk. The greater cream layer volume in raw milk will be secured when it is cooled immediately to 50° F. after milking, bottled and then stored for several hours at 40° F. In pasteurized milk the greatest cream layer volume is secured when the milk is flash cooled to 40° F. or below and stored at 40° F. or below until the creaming has been at least partially completed. W.S.M.

616. Comparative Study of the Bacterial Flora of Grade A and Grade B Milk in New York City. M. L. ISAACS AND M. NUSSBAUM, DeLamar Inst. of Pub. Health, Columbia Univ., New York, N. Y. Am. J. Pub. Health, *30:* 9, 2–22. 1940.

A total of 1,130 samples of Grade A and Grade B raw and pasteurized milk were studied. Laboratory tests used were total counts on old and new standard methods agar; count on blood agar; microscopic count; identification of genera and species, quantitative counts of coliform organisms, clostridia spores, yeasts and molds; and toxicity test on guinea pigs.

Agar plate counts of Grade A milk by Standard Methods gave monthly median values ranging from 22,000 to 300,000 for the raw, and from 450 to 2,400 for the pasteurized product. The range of Grade B milk, by the same methods, was from 160,000 to 890,000 for the raw, and 8,400 to 21,000 for the pasteurized. Use of the newly adopted official agar raised the counts of most samples of milk and increased considerably the number of samples of Grade B milk with counts in excess of the limit allowed by the present Sanitary Code of New York City. From this and other tests, the conclusion is made that Grade A milk is a more uniform and cleaner product than Grade B milk. M.W.Y.

617. The Responsibility of The Milk Producer to the Consumer. W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. The Bimonthly Bull., *25:* 203, p. 31. March–Apr., 1940.

In line with producing clean, safe milk, it is the responsibility of the producer to actively participate in movements to eradicate from herds diseases which can be transmitted through milk. A second responsibility is to make efforts to lower costs of production while still maintaining high standards. In this the producer can be aided by membership in a dairy herd improvement association. Another duty of the producer is to make use of the latest knowledge of the relationship between good feed and high quality milk, from a nutritional standpoint. P.H.T.

618. Essentials for Producing Good Milk and Cream. D. R. THEOPHILUS, Univ. of Idaho., College of Agr., Moscow, Idaho. Ext. Circ., No. 66. May, 1940.

Simple procedures for producing good milk and cream are listed and discussed. Next in importance to having healthy cows is cleanliness of barns, milkers and utensils. Satisfactory methods of cleaning and sterilizing are given. A diagram of a well-planned milk-house is shown as are photographs of proper equipment. P.H.T.

619. Variations in the Percentage of Fat in Cows' Milk. I. E. PARKIN, Pa. State College, School of Agr. and Exp. Sta., State College, Pa., Circ., 222. May, 1940.

In this circular various reasons are given for the fluctuation in the percentage of fat in cows' milk, such as the breed of cows, inherited characteristics of the individual, seasonal variations, effect of time of freshening, and effect of feed and exercise. Mention is made of experimental work being done with drugs and hormones in an effort to increase fat percentages.

P.H.T.

620. Watery Appearance of Frozen Homogenized Milk. G. M. TROUT, Michigan State College, Agr. Exp. Sta., East Lansing, Mich. The Quarterly Bull., p. 10. Aug., 1940.

Experiments were made which showed that homogenized milk freezes slightly more quickly than unhomogenized milk. After the frozen homogenized milk was allowed to melt at 70° F. the milk became snowy or watery. After mixing, however, it appeared as smooth as the unfrozen milk. In general, the higher processing pressures resulted in a more watery appearance when the milk was frozen. The fat of slowly-thawed frozen homogenized milk exhibited marked settling, while that of the rapidly-thawed milk showed the tendency to a lesser extent. For this reason fat analyses of the upper portion of slightly frozen homogenized milk may not give an accurate test of the fat percentage. P.H.T.

621. Is One Grade of Milk Desirable? Editorial, Am. J. Pub. Health, *30:* 1111–1112. 1940.

The new regulations for milk in New York City are discussed. On September 1, 1940, the former Grade A and Grade B pasteurized milks were superseded by one grade of pasteurized milk designated as "Approved" with intermediate standards. Certified milk may still be sold.

Bacteriological requirements for the new milk supply before pasteurization are 150,000 per cc. in the country and 400,000 per cc. in the city, in contrast to 100,000 per cc. and 200,000 per cc., respectively, for Grade A. After pasteurization, the standard is the same, 30,000 bacteria per cc. The cooling temperature for the "Approved" milk is 60° F. as against 50° F. for the former Grade A, and the age limit for the sale of the new grade is 48 hours, instead of the 36 hours for Grade A. A cover cap is required for the new grade. The minimum butter fat is set at 3.3 per cent. Whether the new regulations for milk in New York City are an improvement or not is a matter that has aroused much controversy. M.W.Y.

622. Automatic Control of Pasteurization. Advantages and Safeguards. A. W. FUCHS, U. S. Public Health Service, Washington, D. C. Am. J. Pub. Health, *30:* 477–482. 1940.

Safeguards of design and operation that must be required to insure proper pasteurization in automatic equipment are described and discussed.

Experience with the phosphatase test has indicated the superior reliability of automatic over manual pasteurization. While the safeguards that have been incorporated in automatic pasteurizers are still imperfect, they have been recently improved so as to eliminate many of their weaknesses; on the other hand, the faults inherent in the human element of manual pasteurization are still present. M.W.Y.

623. A Study of Concentration and Freezing as a Means of Preserving Fluid Whole Milk. R. T. Corley and F. J. Doan. Food Res., *5:* 369. 1940.

Pasteurization temperature of 82.2° C. (180° F.) were found preferable to lower temperatures. Homogenization after condensation retarded the development of tallowy flavor and coagulation during storage as did the higher pasteurization temperature. Such milk could be stored frozen for fifteen weeks and be reconstituted to normal milk showing *in vitro* digestion characteristics superior to boiled milk and somewhat similar to evaporated and acidified milk. Milk contaminated with copper develops tallowy flavor unless storage period is less than five weeks. P.A.D.

624. High Temperature Short Time Pasteurization. H. D. Kay. Milk Industry, *21:* 2. Aug., 1940.

The author discusses the history of the high temperature short time pasteurization in Great Britain. The results of several installments of short time high temperature pasteurizers operating at different temperatures and for different periods of exposure are given. At the present time high temperature short time pasteurization does not meet the legal requirements of pasteurization in England. W.J.C.

PHYSIOLOGY

625. Studies on the Secretion of Milk Fat. 3. The Effect of Thyroxine Administration on the Blood Lipoids and on the Nature of the Milk Fat. James A. B. Smith and Noshir N. Dastur, Hannah Dairy Res. Inst., Kirkhill, Ayr. Biochem. J., *34:* 1093. 1940.

The administration of thyroxine to cows markedly increased the yields of milk and milk fat. There was, however, no consistent change in the level of the non-fatty solids of the milk except for a slight decrease in the protein concentration.

There was no alteration of any importance in the actual nature of the milk fat during the period when its yield was enhanced except for a slight temporary change at the beginning of the hyperthyroid period. The concentration of sugar in the plasma was increased by some 10–26 per cent during the period of hormone administration and this was accompanied by

a general decrease of 10–20 per cent in the concentration of the main lipoid constituents. V.C.S.

MISCELLANEOUS

626. Locker Plant Operation. K. F. WARNER, extension meat specialist, U. S. Dept. of Agr. Ice Cream Field, *36:* 3, 12. Sept., 1940.

Ten years ago there were only a few dozen food locker plants in 3 or 4 states, while today there are over 2800 locker plants in 44 states. Over 75 per cent of these plants are operated in connection with some other business, such as an ice cream plant, a creamery or an ice plant.

Lockers of about 250 pounds capacity rent for from $5.00 to $15.00 a year, with or without additional service and service charges for preparing, packaging and freezing foods.

It is claimed that locker plants can operate successfully with from 100 to 150 lockers if operated in connection with some other business but where plants operate independently 300 to 600 or more lockers are customary. Construction costs vary from $25.00 to $40.00 per locker.

The author points out that many farm families are able to produce their own food but they are not equipped to preserve it, thus frozen food lockers make it possible to supplement the ordinary diet of canned and salted foods with fresh home-grown beef steaks during the summer and fresh frozen chicken and strawberries in the winter. Many city families also find uses for frozen food lockers, but families who produce most of their food are better potential customers than families accustomed to buying most of it.

The following disadvantages of locker service are listed: (1) Need of cash outlay when the locker is rented and the food purchased, (2) Foods may deteriorate in these lockers unless properly handled, and (3) There is no delivery service.

The importance of properly packaging and freezing suitable quality products is emphasized and the necessity of proper construction and insulation stressed. The question of selecting the most useful type of locker equipment also deserves serious consideration. It is finally stated "Locker plants and locker service are merely new efforts in the continuing process of adapting refrigeration to family needs." W.C.C.

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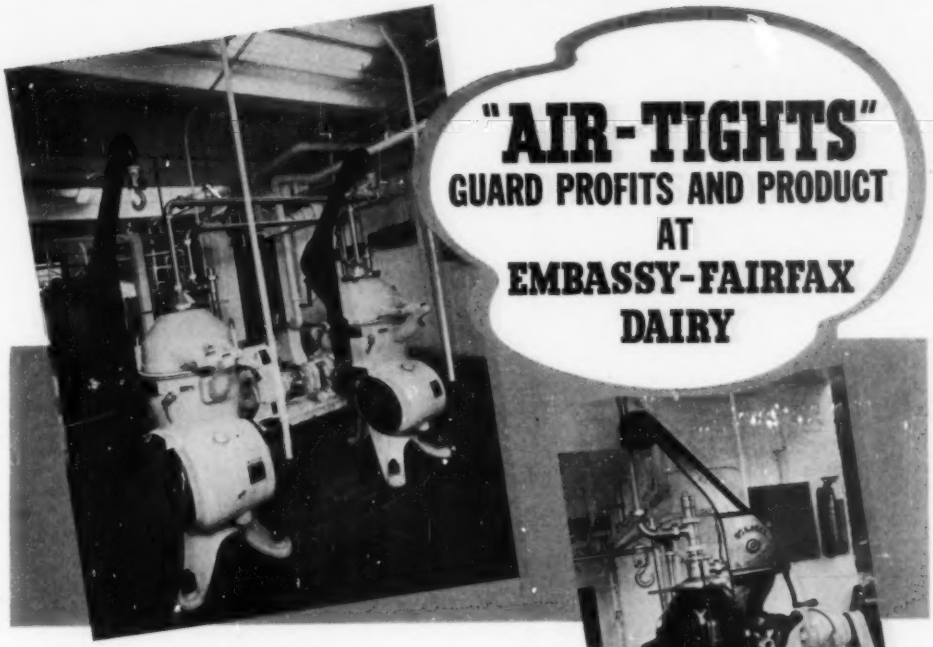

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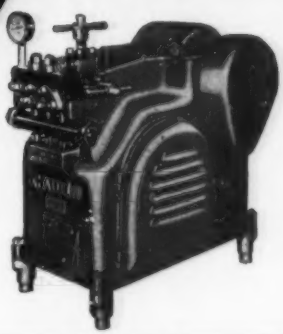

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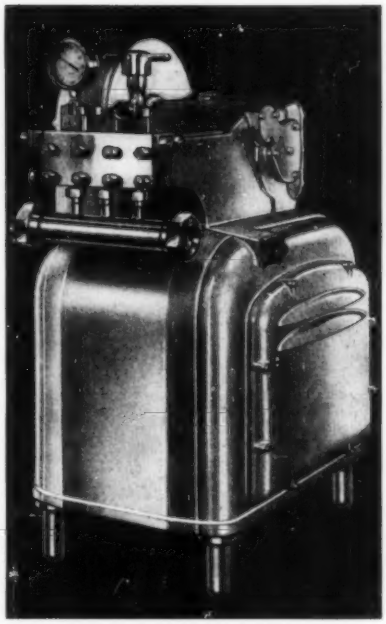

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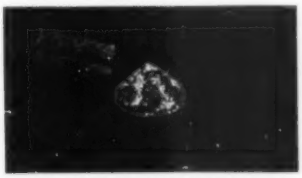

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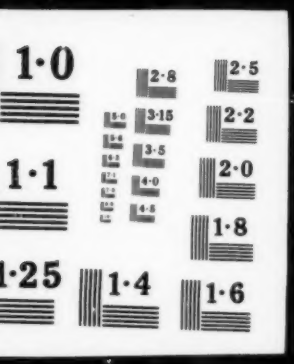
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pressed in terms of the lines per millimeter recorded by a particular
ls in chart indicate the number of lines per millimeter in adjacent

termine the reduction ratio and multiply the number of lines in the
lines recorded by the film. As an aid in determining the reduction
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mple: the line is 20 mm. long in the film image, and 100/20 = 5.

in the film with microscope, and note the number adjacent to finest
ltiply this number by the reduction factor to obtain resolving power
roup of lines is clearly recorded while lines in the 10.0 group are
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